Cytokine Crosstalk in Inflammatory & Mechanical Stress Mechanisms in Osteoarthritis

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WHERE THE WORLD COMES TO GET BACK IN THE GAME

Michelangelo's David: the late years

OA Risk factors:

-Mechanical factors

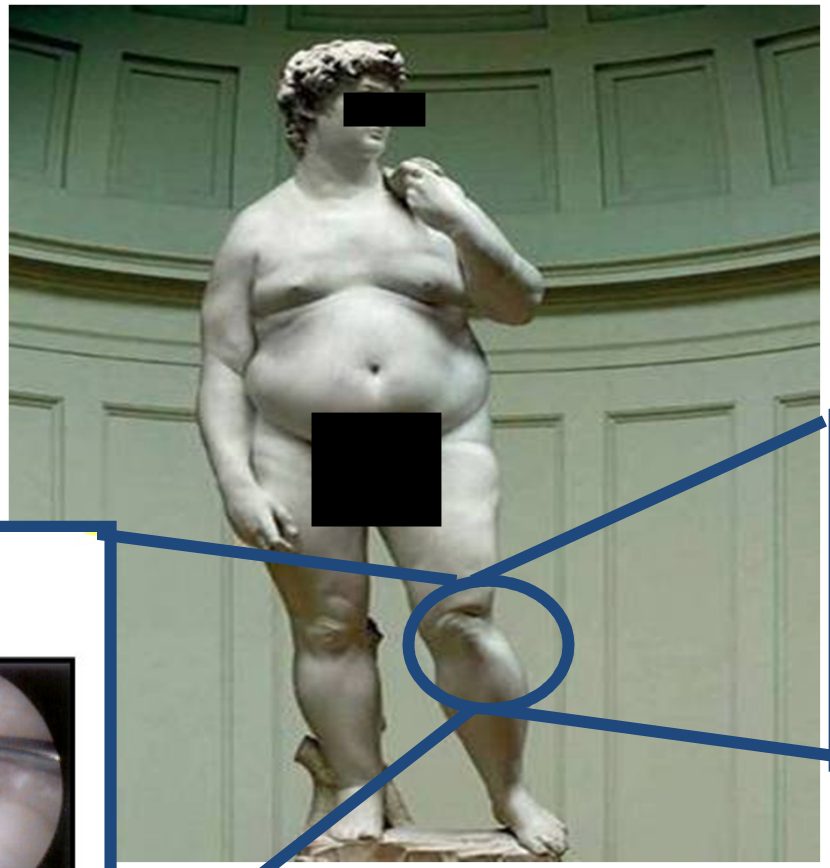
-Injury

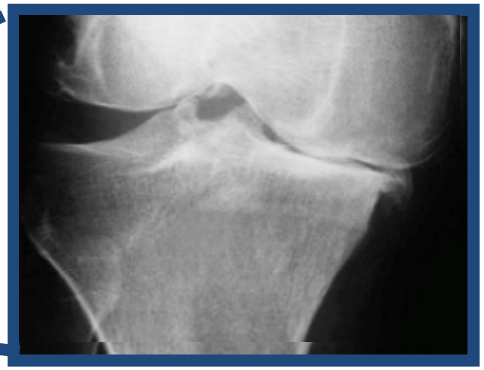
-Age

-Genetics

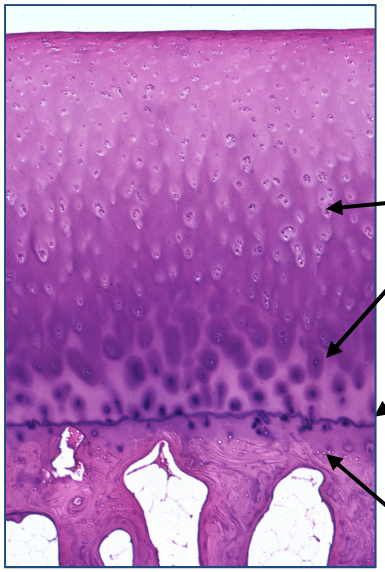
-Obesity

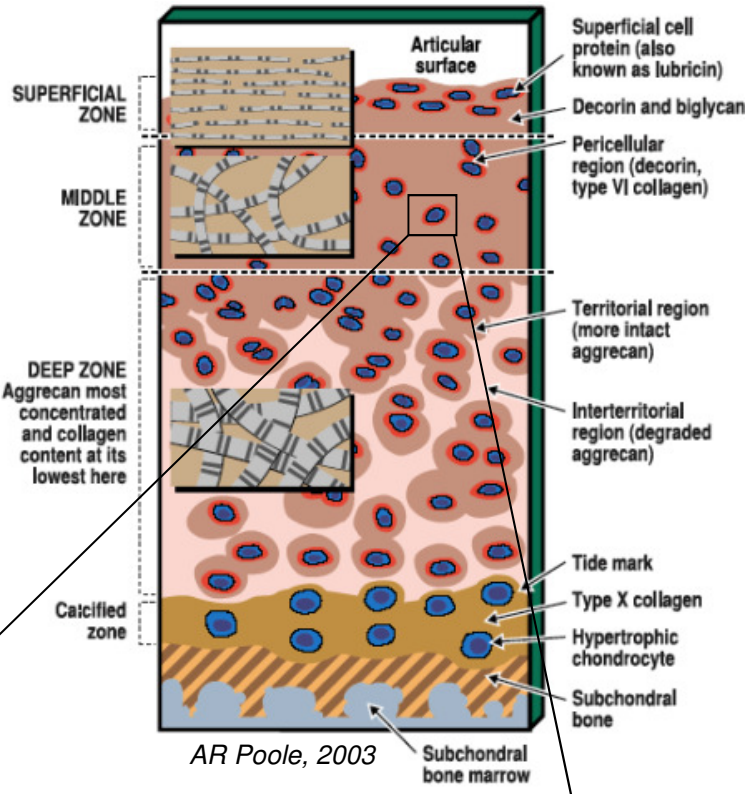
-Inflammation



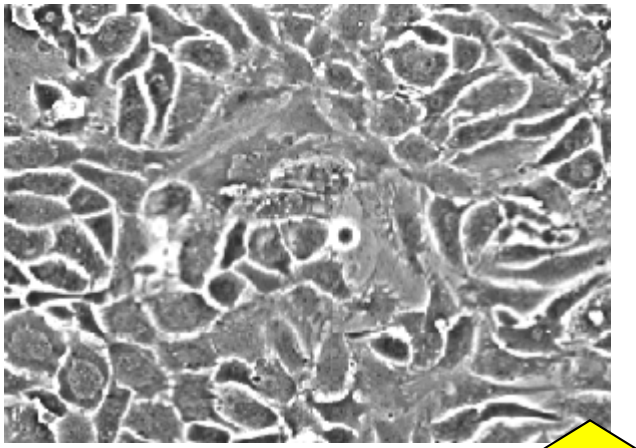


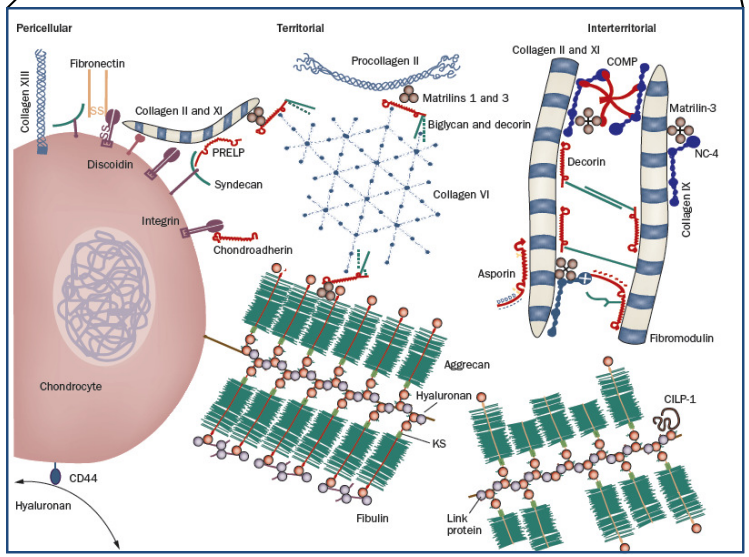
Normal articular cartilage





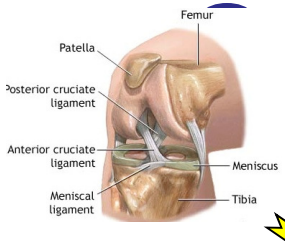
AR Poole, 2003

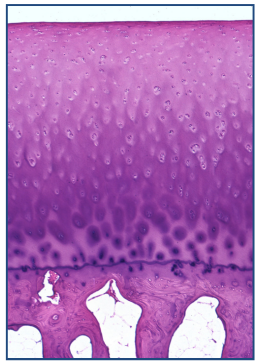




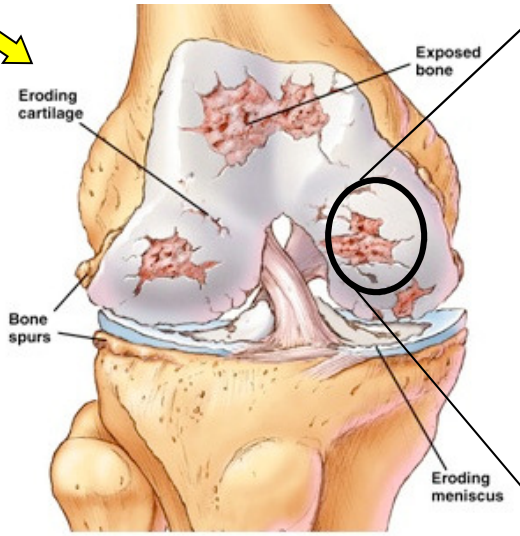
Heinegard & Saxne: Nature Rev Rheumatol 7:50-56, 2011

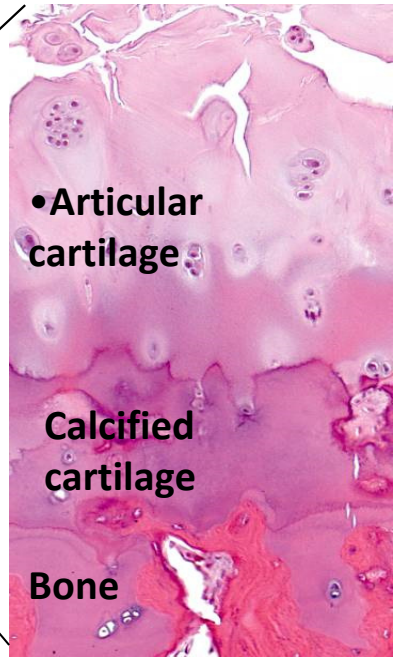
steoarthritis





normal





•Fragmentation and fissuring of cartilage matrix

•Local chondrocyte proliferation and cell death

•Increased cartilage degrading activity and altered synthetic activity

•Cartilage calcification and tidemark advancement

•Vascular invasion from subchondral bone

Whole joint disease

-Articular cartilage (*degeneration/loss)*

-Calcified cartilage

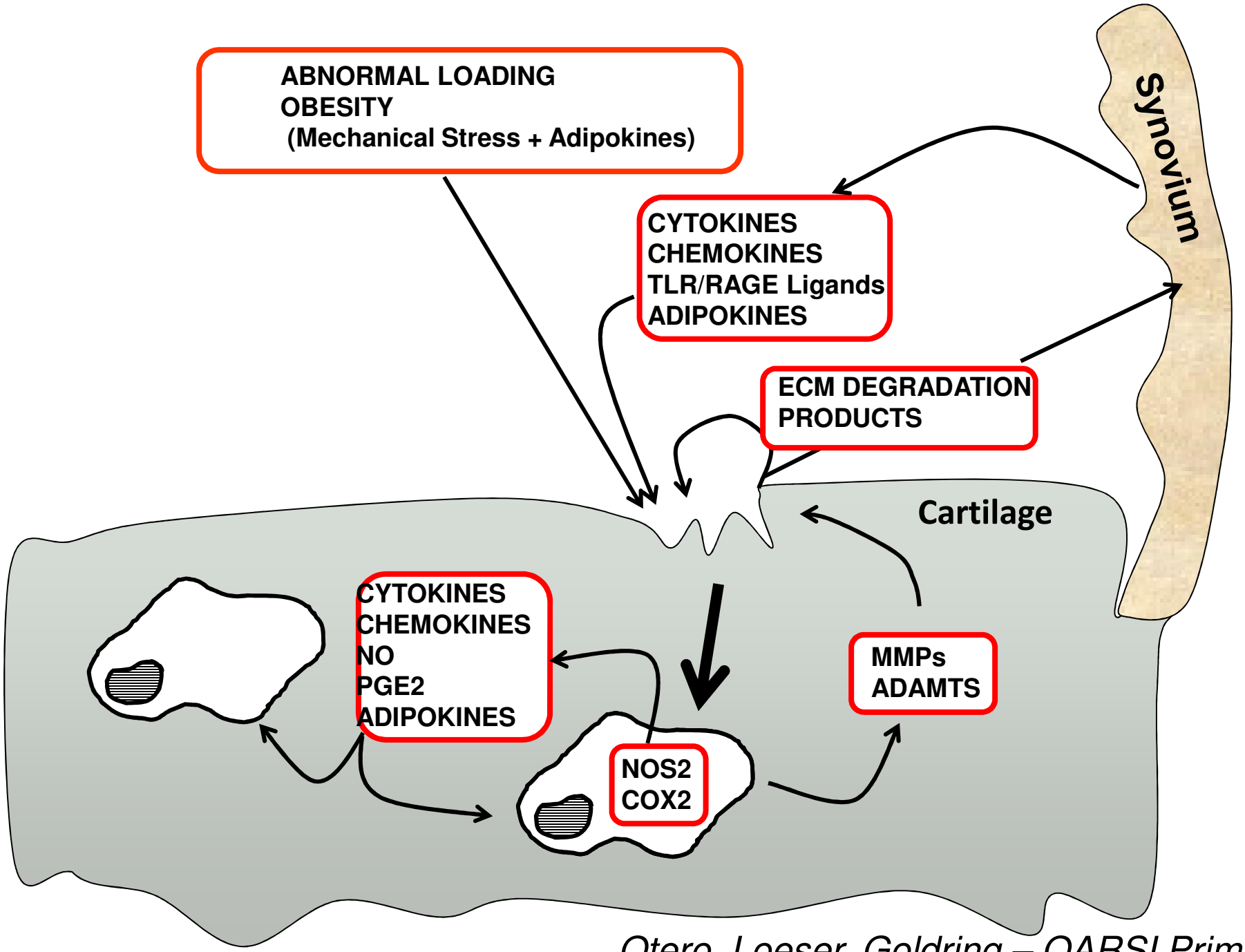
-Bone (*osteophytes, subchondral sclerosis)*

-Meniscus, ligament, tendon (*disruption)*

-Synovium (*synovitis)*

•The complexity of the composition and cellular organization of articular cartilage presents a tissue engineering challenge

• Successful therapies must prevent damage or promote repair to recapitulate the physiological and functional properties of cartilage.

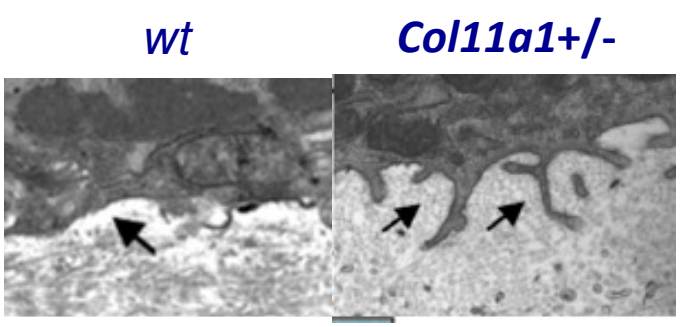


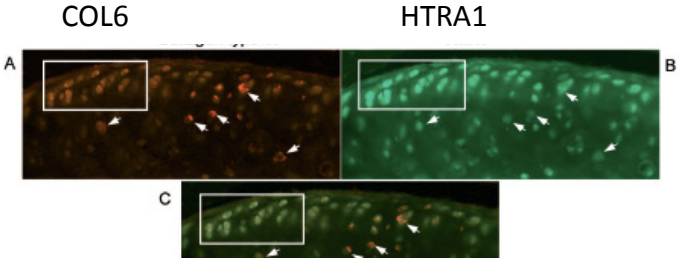
Otero, Loeser, Goldring – OARSI Primer

Major Points

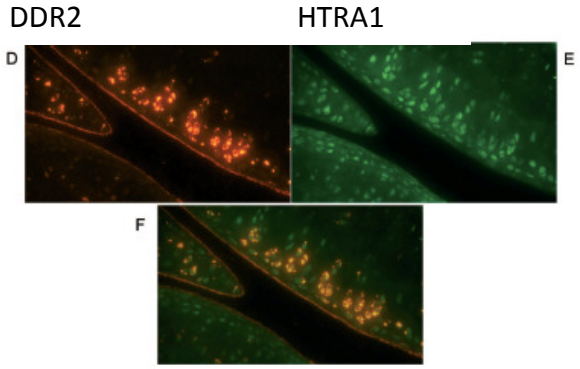
- Complexity of normal cartilage matrix and lack of adequate repair mechanisms once OA progresses
- Pivotal role of the matrix metalloproteinase, MMP-13, in OA progression as collagenase that preferentially degrades type II collagen
- Similarity of signaling pathways induced by mechanical and inflammatory stress
- Heterogeneity of OA phenotypes, but with common markers over the time course of initiation and progression

Role of pericellular matrix & discoidin domain receptor (DDR) 2

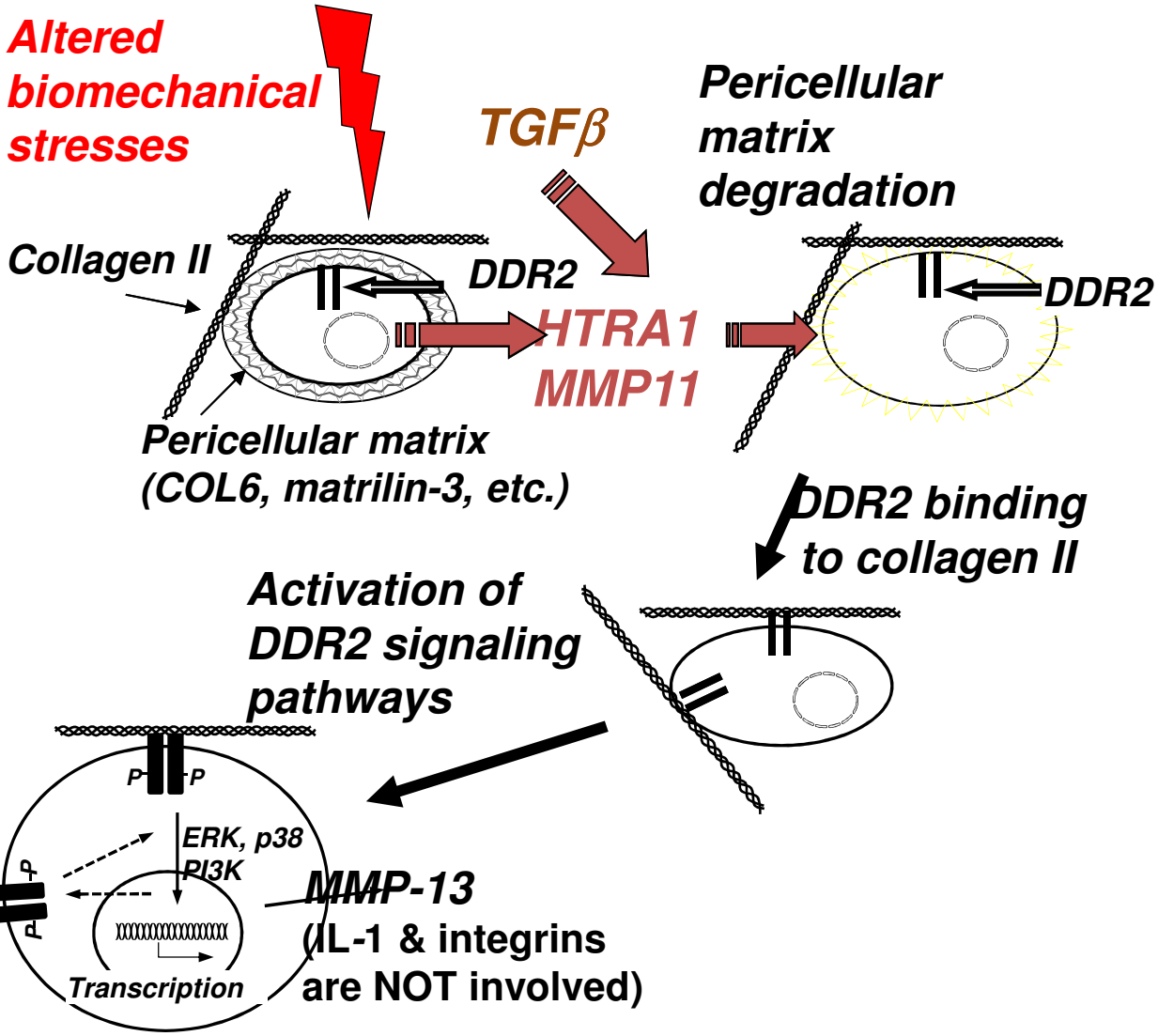




COL6/HTRA1



DDR2/HTRA1

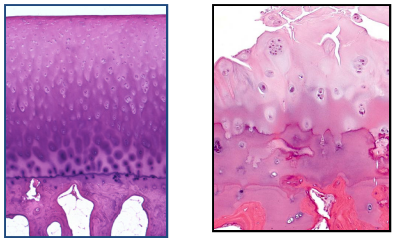


Xu L (Yefu Li) et al. Am J Pathol 2011;179:1338-1346

Strategies for identifying and characterizing mediators in osteoarthritis (OA)

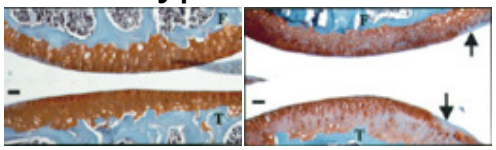
Clinical material

Normal OA



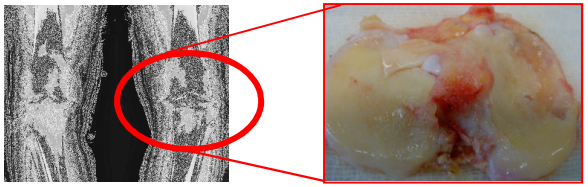
In situ hybridization
Immunohistochemistry

Wild type OA



Gene & protein expression

In vivo mouse models

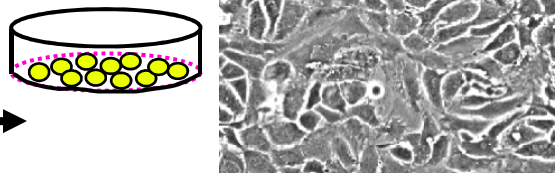


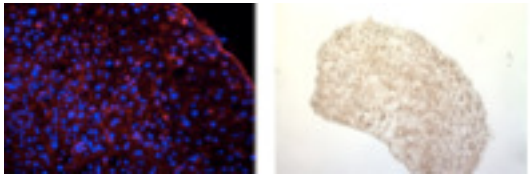
Tibial plateau-OA patient-total knee replacement

- Trauma
- Aging
- Genetics
- Inflammation

In vitro culture models

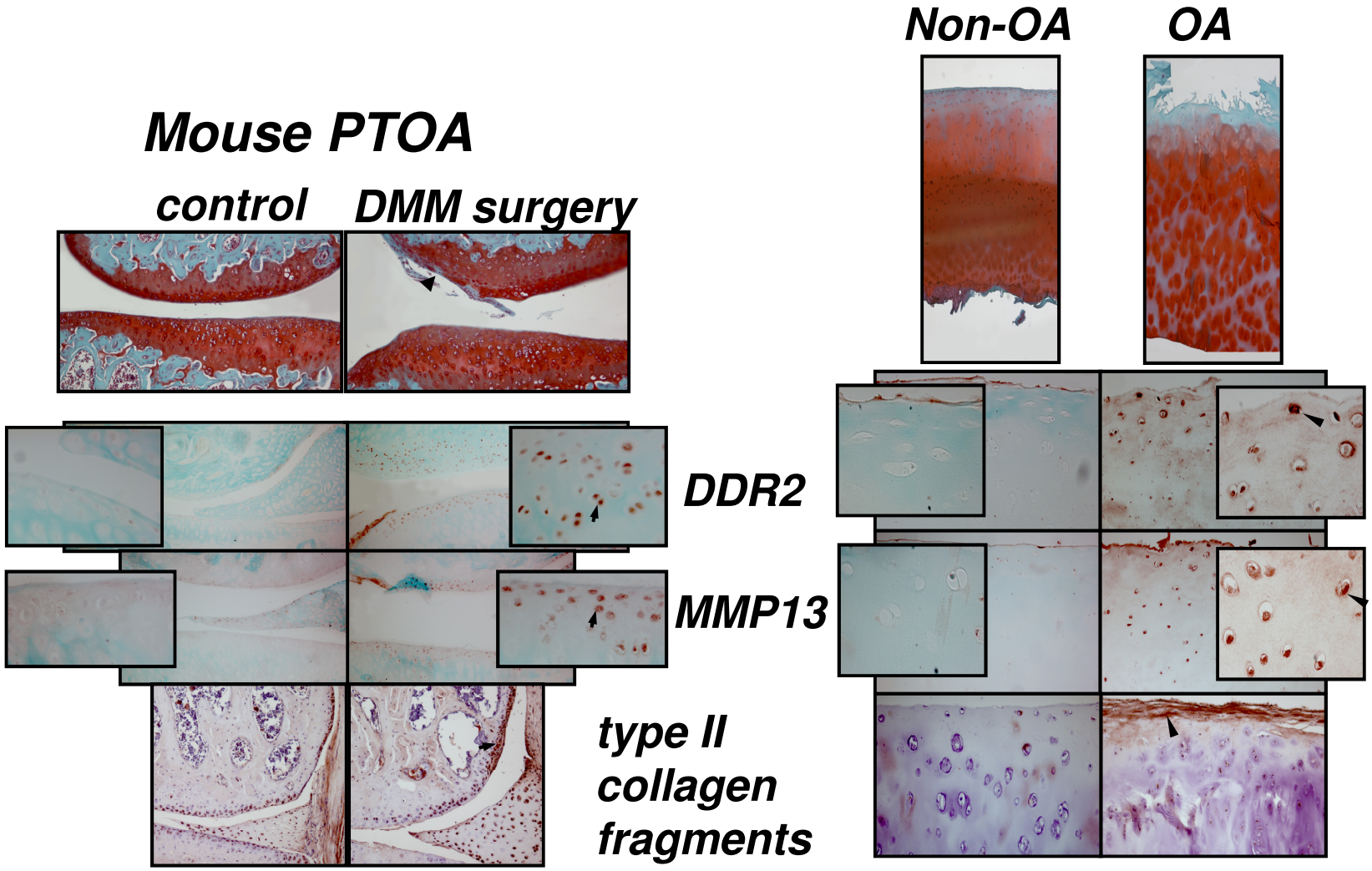
- Cytokines
- Mechanical stimulation





MMP-13
=collagenase
preferentially degrades type II collagen

SIMILAR RESPONSES IN MOUSE & HUMAN OA



Xu et al., Arthritis Rheum 2007

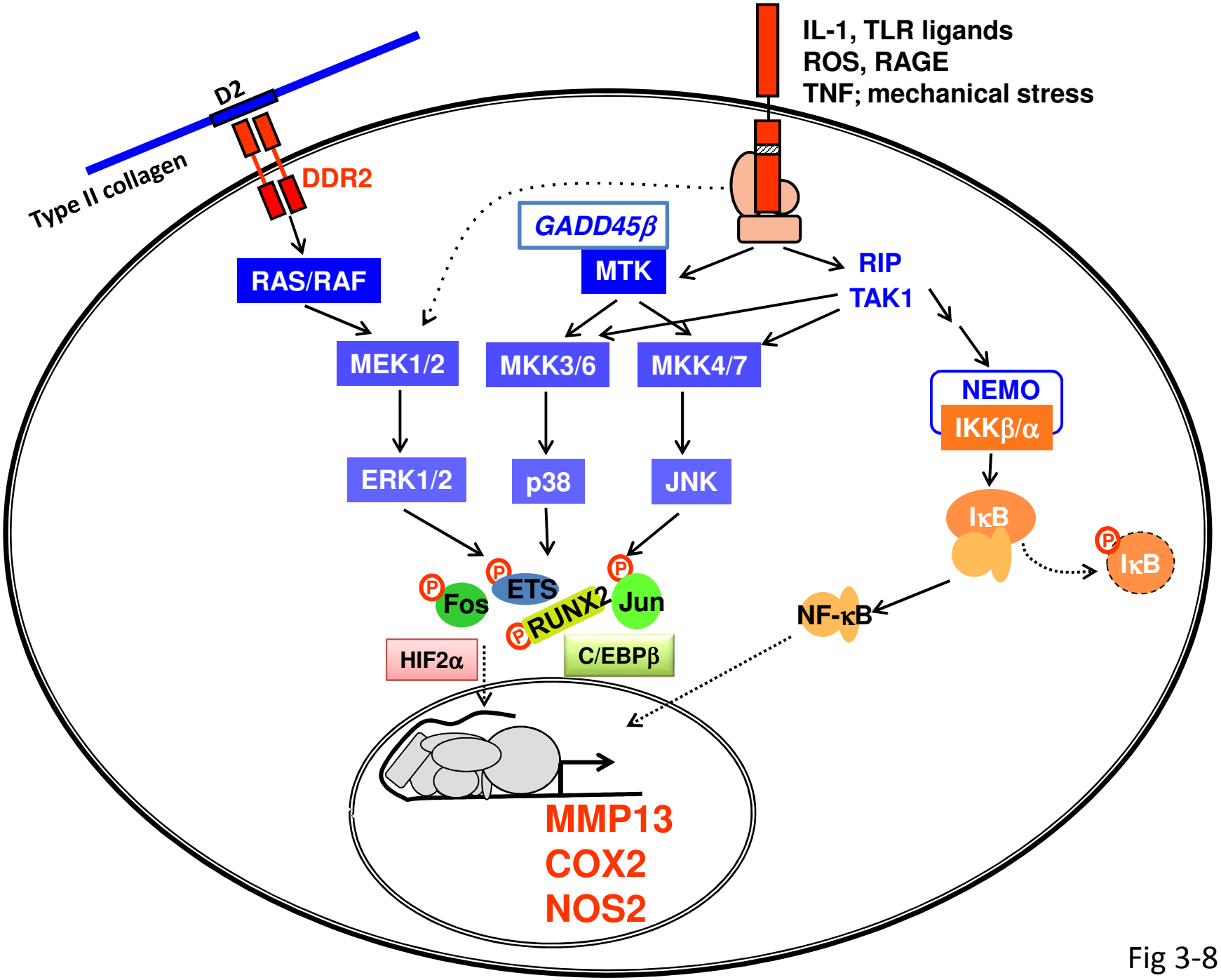
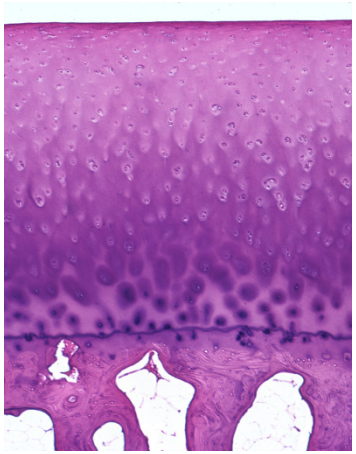

IL-1, TLR ligands
ROS, RAGE
TNF; mechanical stress
D2
DDR2
Type II collagen
GADD45β
MTK
RIP
TAK1
RAS/RAF
MEK1/2
MKK3/6
MKK4/7
NEMO
IKKβ/α
ERK1/2
p38
JNK
IκB
IκB
Fos
ETS
RUNX2
Jun
NF-κB
HIF2α
C/EBPβ
MMP13
COX2
NOS2

Fig 3-8

From homeostasis to osteoarthritis

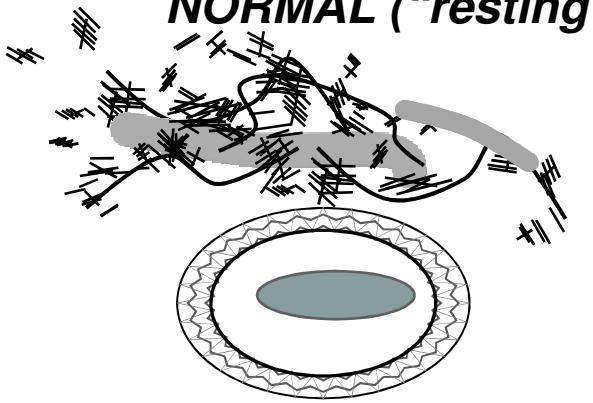


Articular cartilage

Calcified cartilage

Subchondral trabecular bone

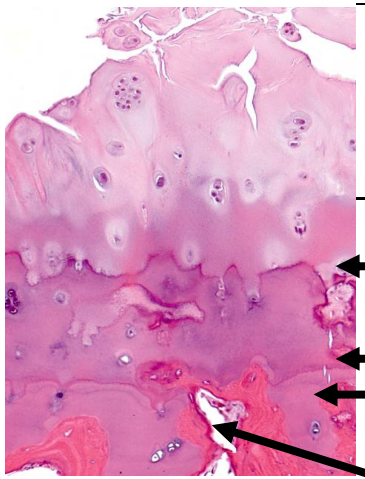
NORMAL ("resting")



Mechanical and Inflammatory Stress

Phenotypic Modulation

OA ("activated")

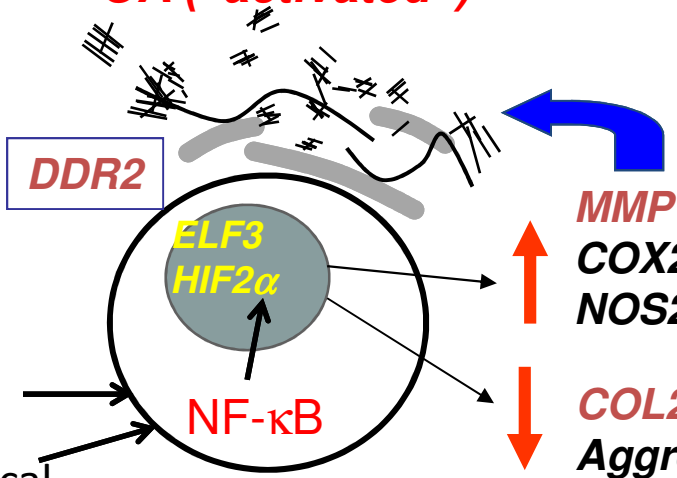


Articular cartilage

Tidemark duplication

Subchondral cortical bone

Vascular invasion

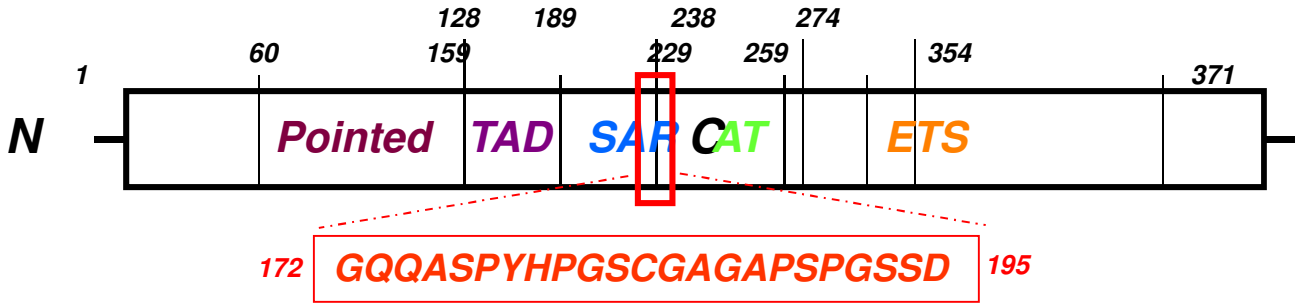


Cytokines
(IL-1β, TNFα)

Mechanical Stress

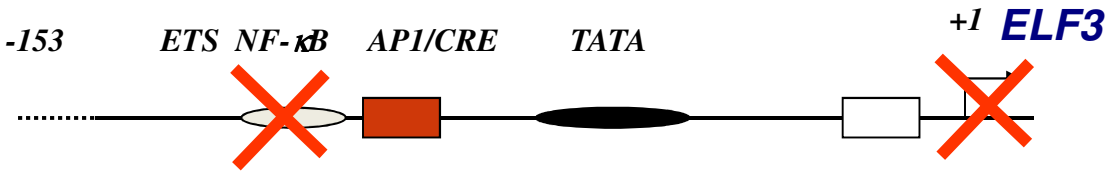
Adapted from Marcu, Goldring et al., Curr Drug Targets 11: 599-613 (2010)

ELF3 gene transcription is dependent on NF-κB



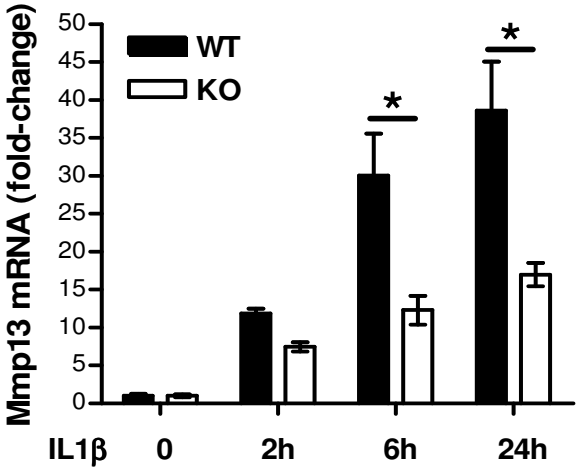
ELF3B

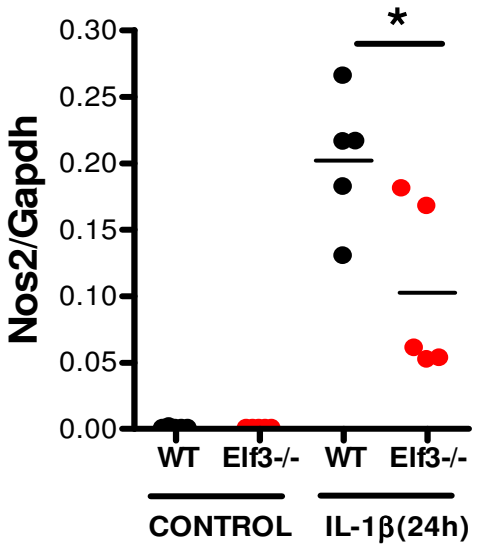
- ***Also known as Epithelial Specific ETS factor 1 (Ese-1), ETS related transcription factor (Ert), epithelial restricted with serine box (Esx) and Jen.***
- ***Roles in epithelial cell differentiation, gut development, apoptosis, transformation, tumor invasion and inflammation*** *(Oettgen, 1997; Trojanowska, 2000; Ng, 2002; Cabral, 2003; Reddy, 2003; Prescott, 2004; Peng, 2008, Wu, 2008; Lee, 2008)*
- ***Induced by inflammatory cytokines (IL-1β, TNF-α) and LPS via NF-κB; activates NOS2, COX2 and MMP13 transcription.***
- ***Mutation of the NF-κB site in the ELF3 promoter blocks induction of promoter activity.***

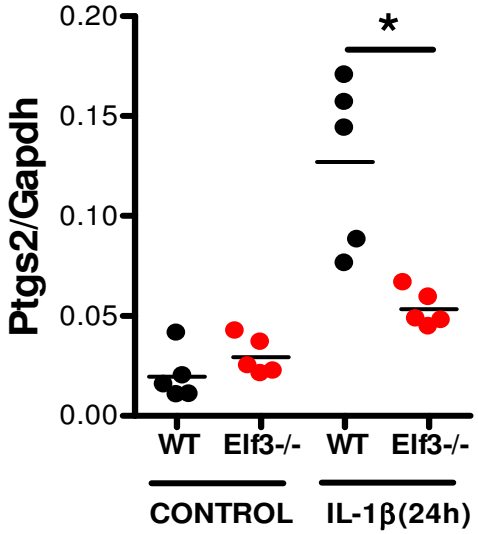


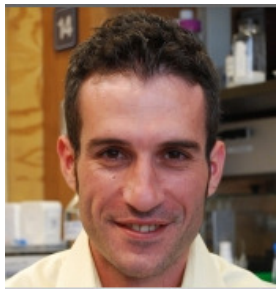
Elf3 mediates inflammatory actions in chondrocytes

- Elf3 deletion decreases the induction of Mmp13, Nos2 and Ptgs2 (Cox2) mRNA levels upon stimulation with IL-1β.



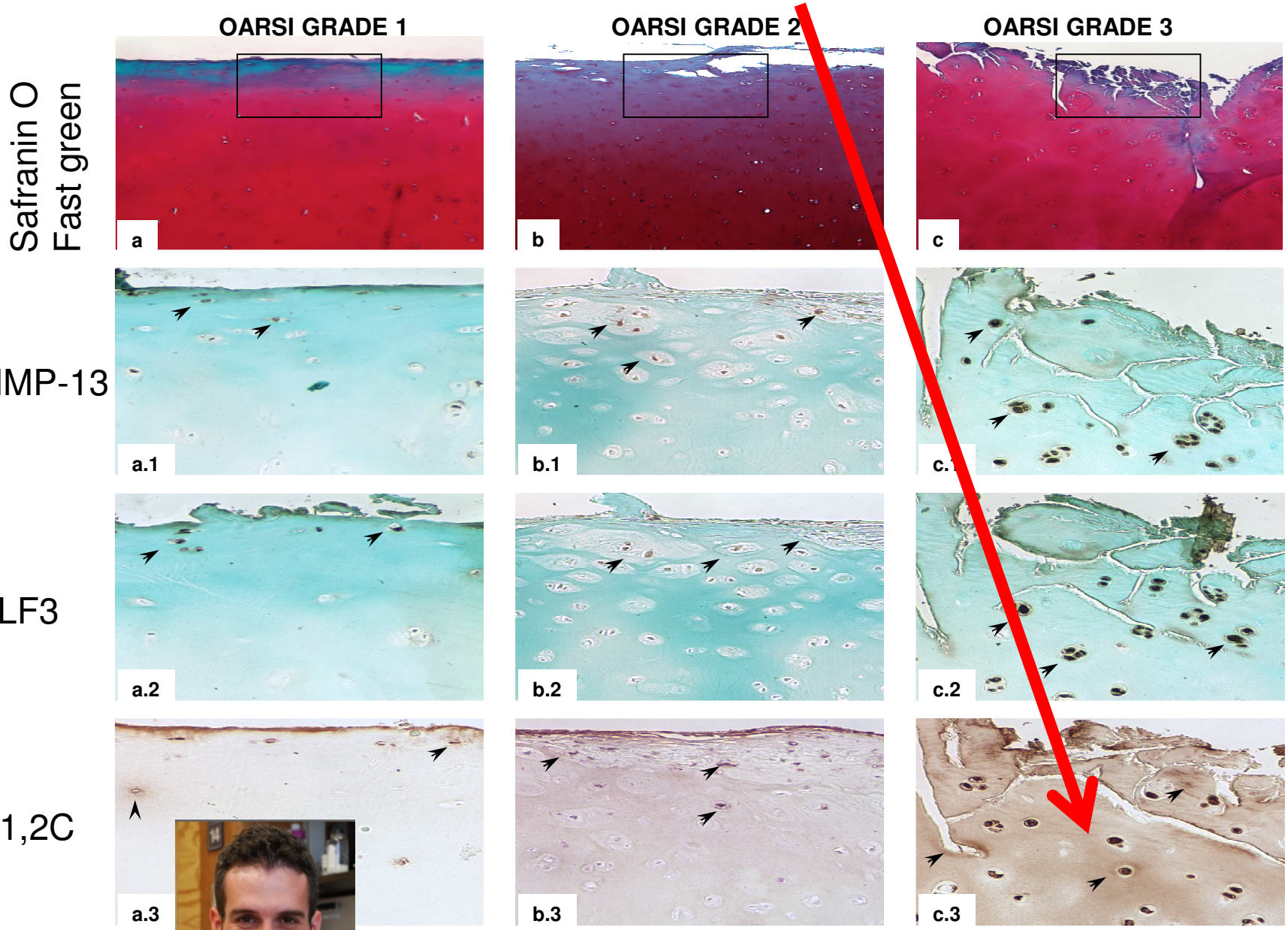






Miguel Otero et al., J Biol Chem 2012

MMP-13, ELF3, and increased collagen degradation (C1,2C)



Miguel Otero et al., J Biol Chem 2012; 287:3559-3572

Cartilage-specific deletion of Elf3

–>Attenuation of OA in surgically induced (DMM) model?

COMP-driven (Tet-OFF) overexpression of Elf3 in synovium and cartilage

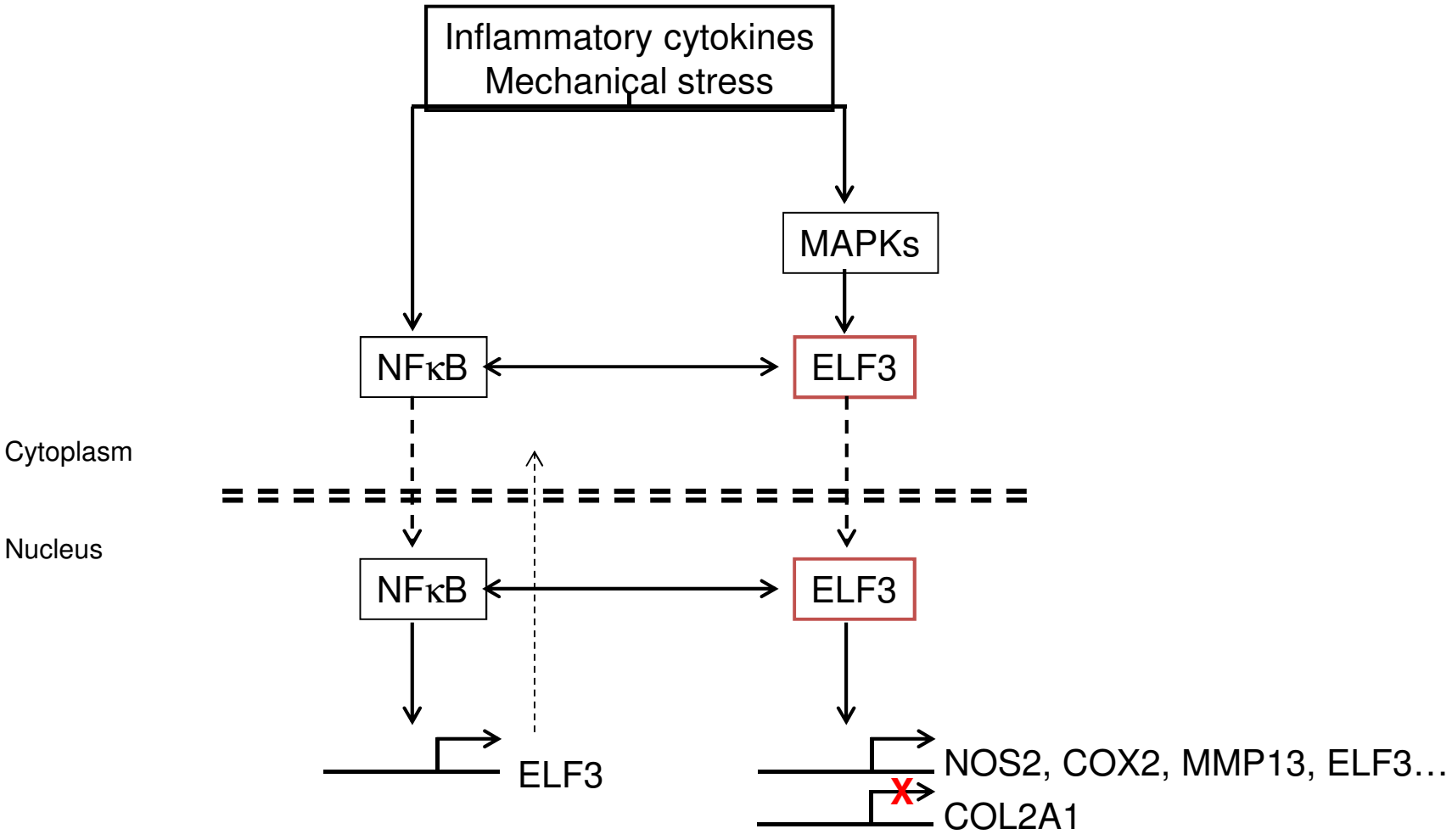
–>spontaneous OA or exacerbation in DMM model?

Poster #452

Elisabeth Wondimu,
Miguel Otero, Darren Plumb, et al.

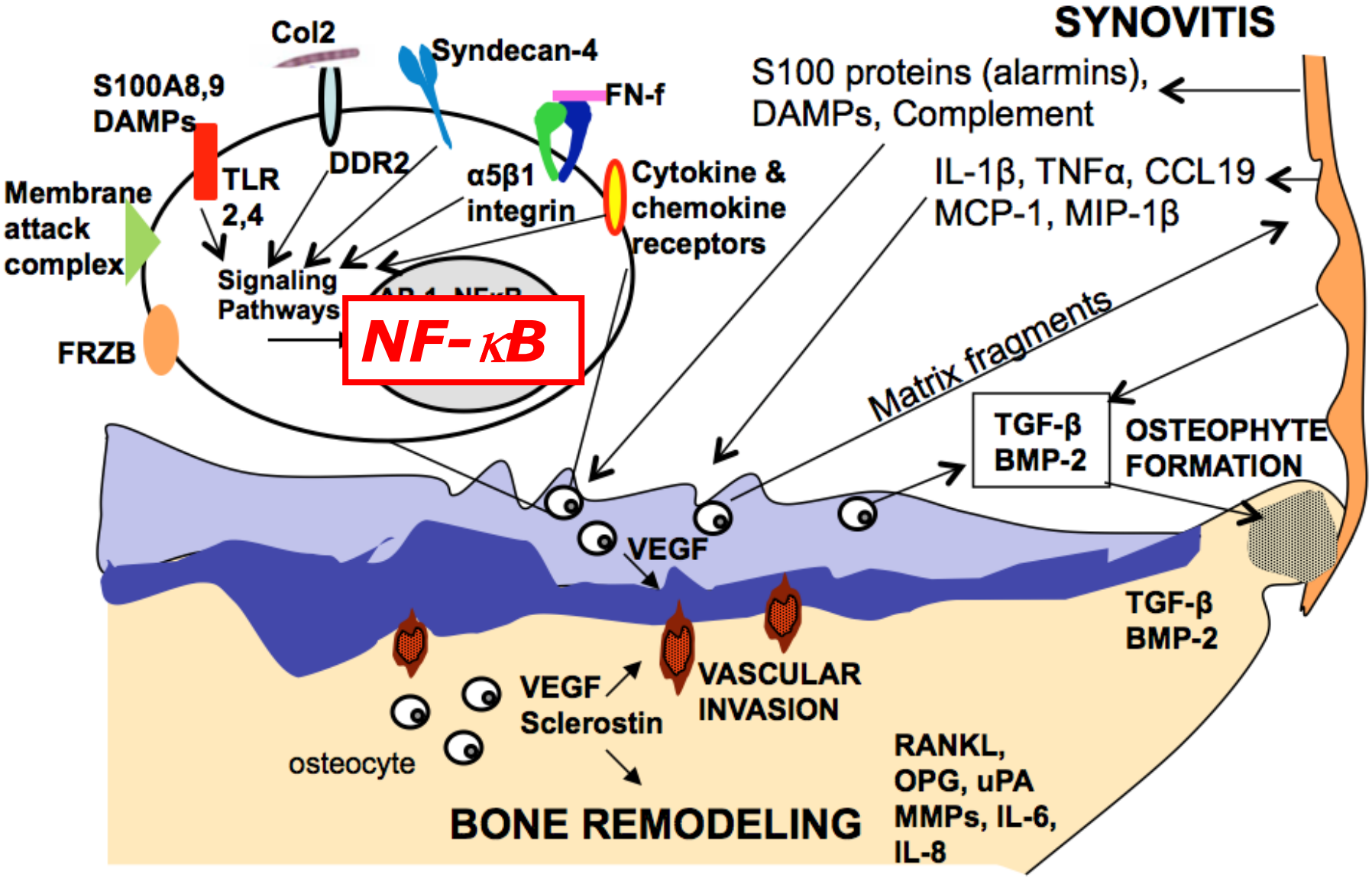
Supported by the
National Institutes of Health

Elf3: upstream and downstream



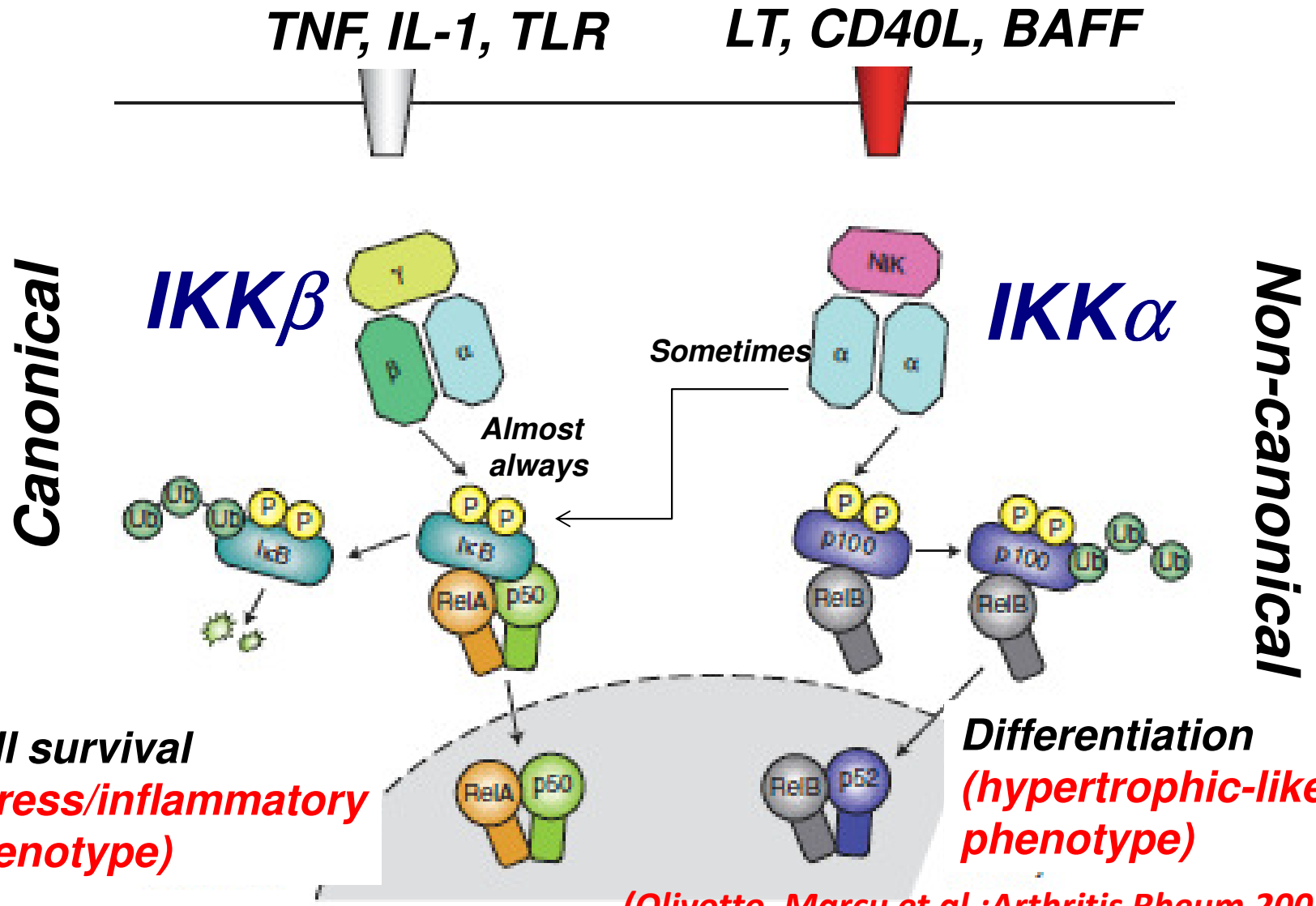
Sharrocks 2001; Rudders 2001; Eckel 2003; Grall 2005; Kopp 2007; Wu 2008; Peng 2008; Otero 2012; Longoni 2013

Selected factors involved in the osteoarthritic process in the synovium, cartilage, and bone



R. Loeser, S. Goldring, C. Scanzello & M. Goldring, Arthritis Rheum 2012

IKK signalosomes activate distinct NF-κB pathways

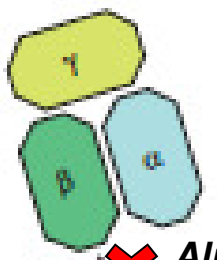


(Olivotto, Marcu et al.:Arthritis Rheum 2008)

Lawrence & Goretsky: Cold Spring Harb Perspect Biol, 2009

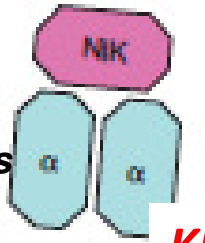
Differential requirements for IKKβ and IKKα in chondrocyte differentiation

- Human OA primary chondrocytes
- shRNA knockdown of IKKα and IKKβ
- 3D/pellet cultures, 1-3 weeks



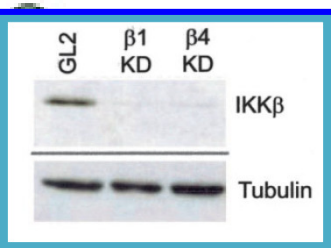
Almost always

Sometimes

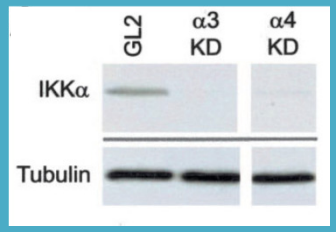


KINASE INDEPENDENT

Canonical



IKKβ KD 'phenotype:
-increased SOX9
-no change RUNX2



IKKα KD 'phenotype':
-decreased RUNX2
-decreased SOX9 expression

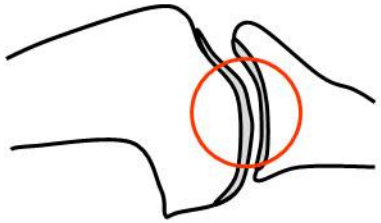
Non-canonical

-Increased GAG (β>α) and type II collagen (α>β)
-Decreased type X collagen (α>β) and mineralization
-Decreased ECM remodeling (C1,2C and DIPEN) independent of MMP13 gene expression levels

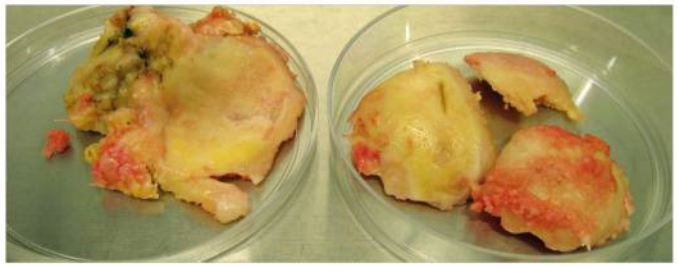
Olivotto (Marcu) et al., Arthritis Rheum 2008 and 2010

In vitro Chondrocyte Differentiation

IKKα(F/F) iMACs

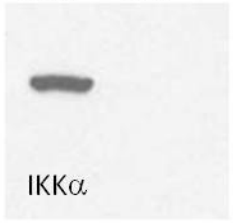


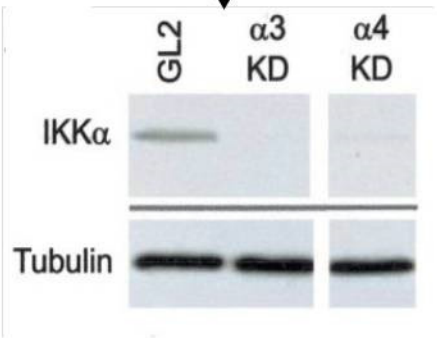
Human OA Primary Chondrocytes

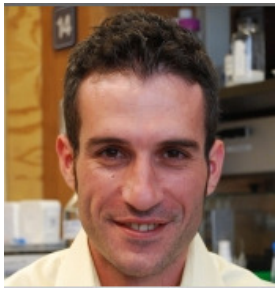


shRNA (anti-IKKα)

Puromycin selection

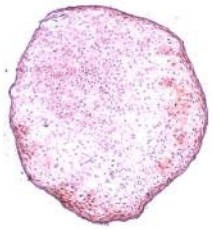


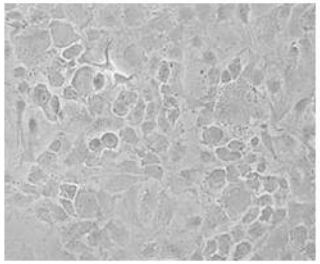




Miguel Otero

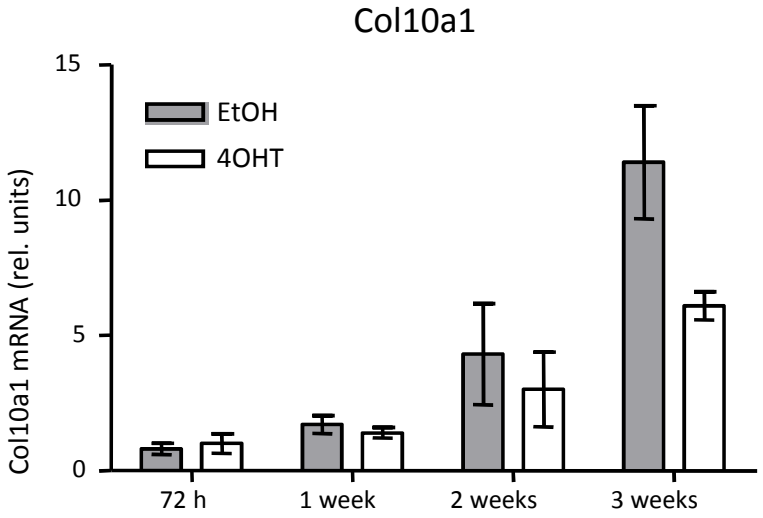
3D/Pellet Cultures and "High-Density" Monolayers

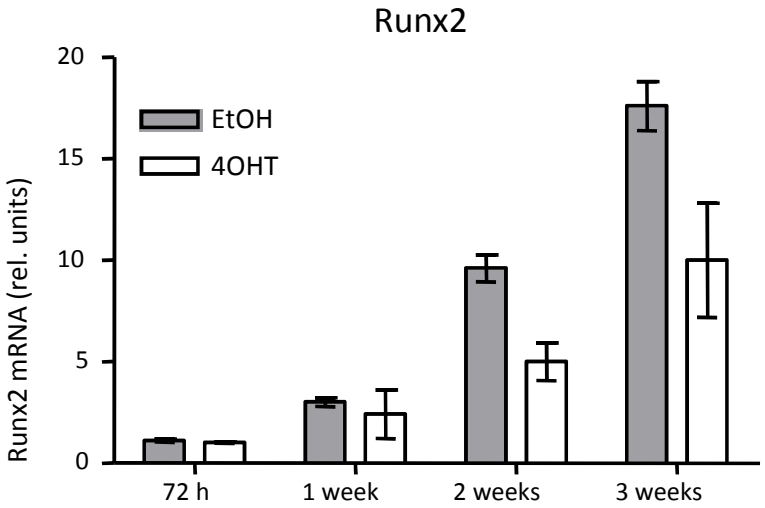


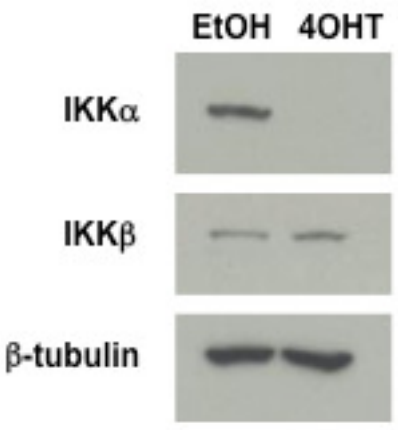


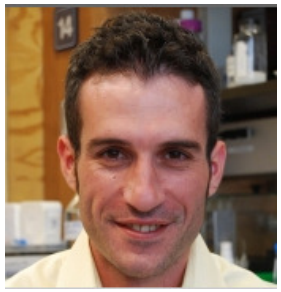
1-3 weeks
+10% FBS
+Ascorbate
(+ITS)

Reduced expression of hypertrophy markers in IKKα KO chondrocytes







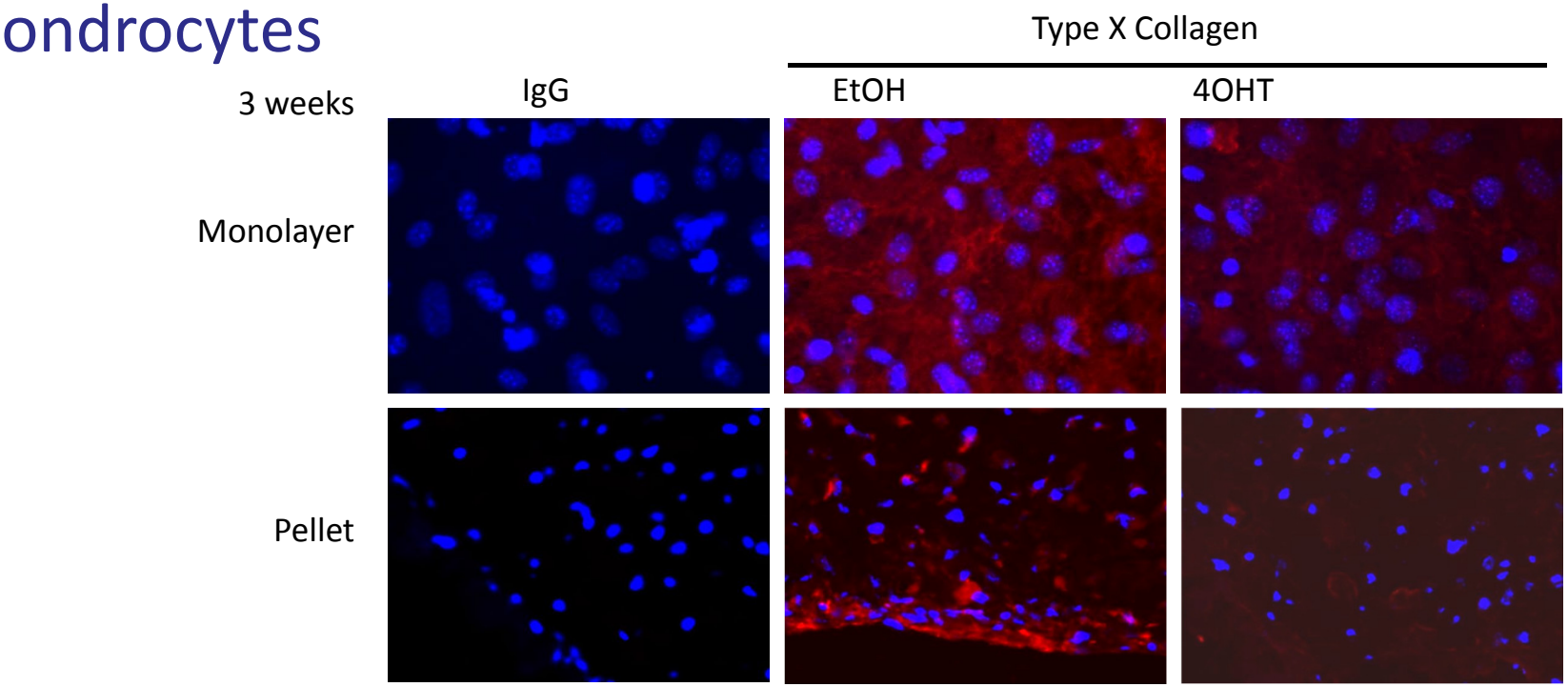


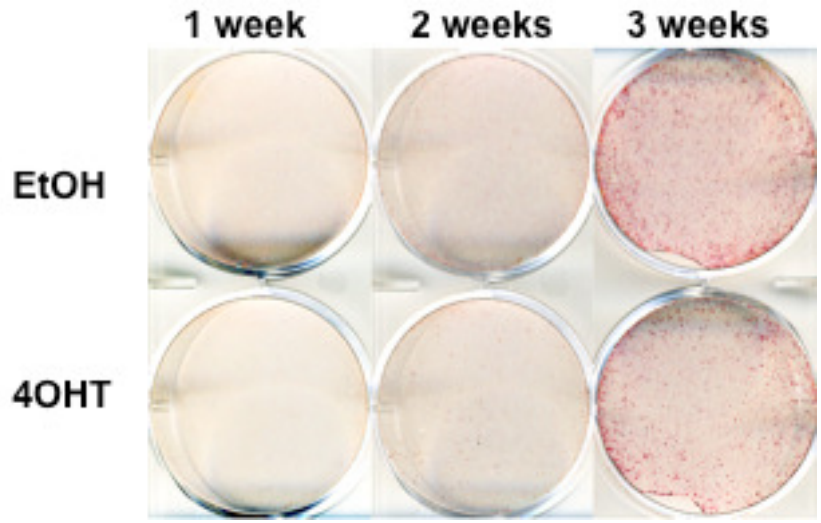
Miguel Otero

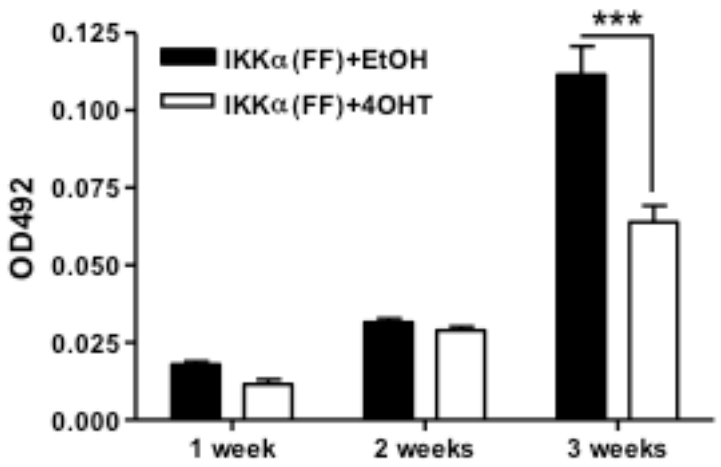
Olivotto E, Otero M, Astolfi A, Platano D, Facchini A, Pagani S, Flamigni F, Facchini A, Goldring MB, Borzì RM, Marcu KB. IKKα/CHUK Regulates Extracellular Matrix Remodeling Independent of Its Kinase Activity to Facilitate Articular Chondrocyte Differentiation. ***PLoS One 2013; 8:e73024***

Eleonora Olivotto

Reduced type X collagen and mineralization in IKKα KO chondrocytes

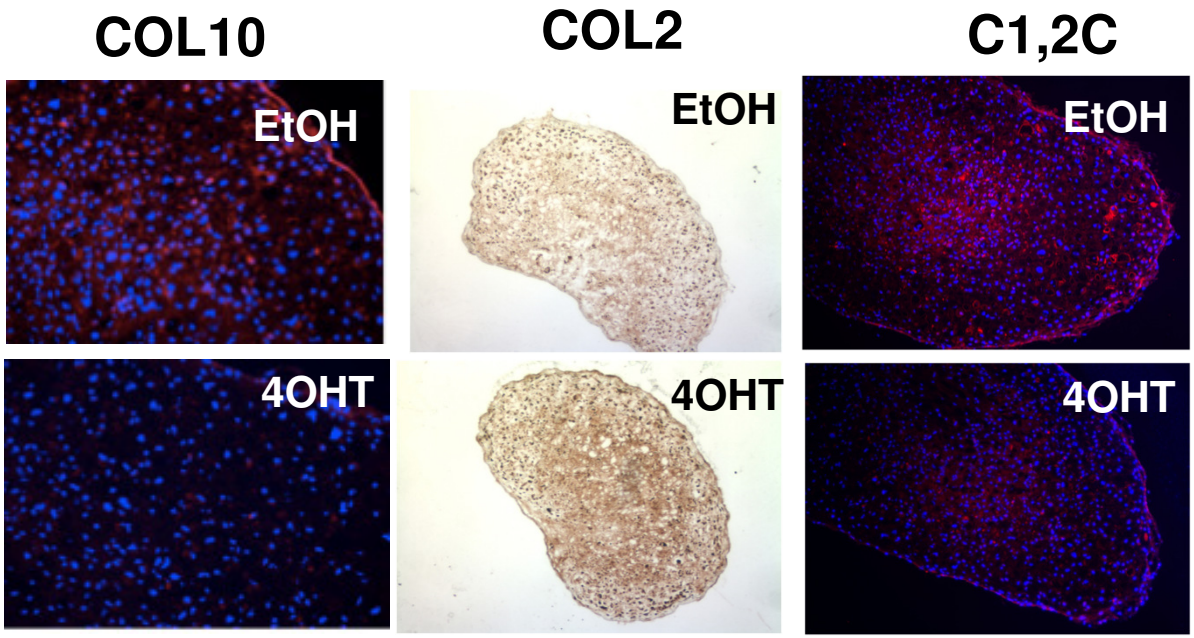


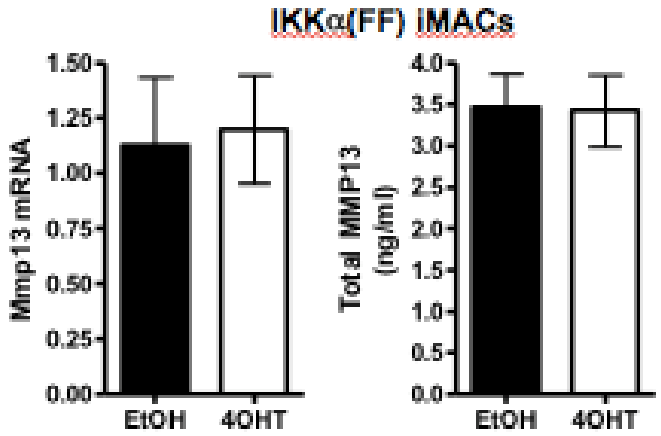


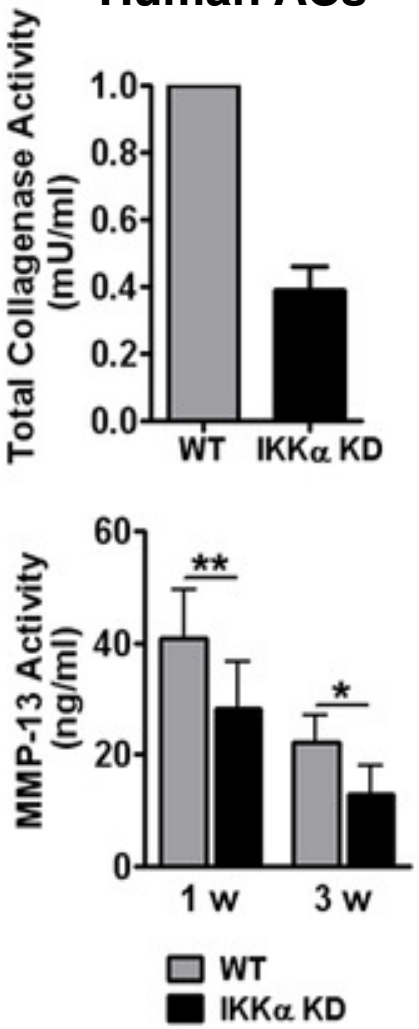


Olivotto, Otero et al., PLoS One 2013

Reduced collagenase activity in IKKα KO orKD chondrocytes is not dependent on MMP-13

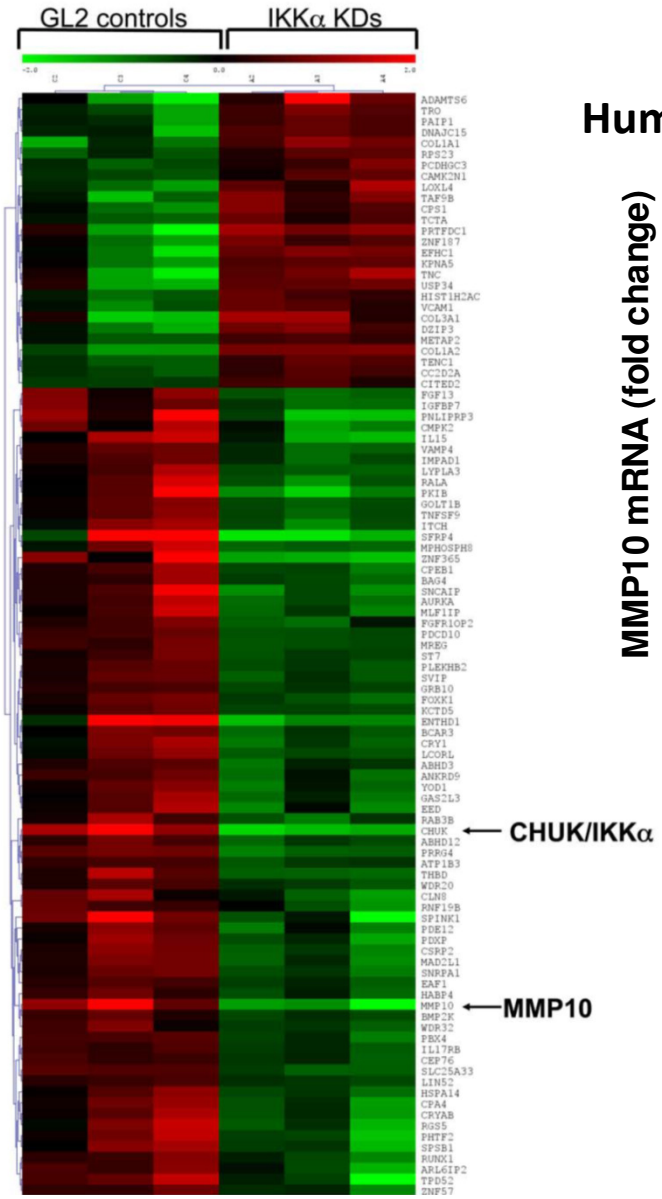


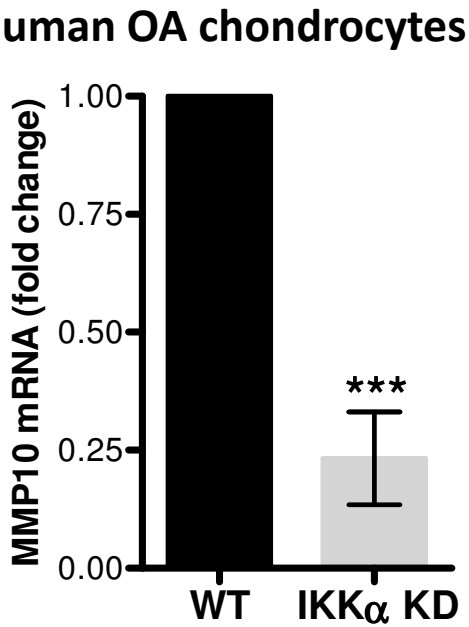


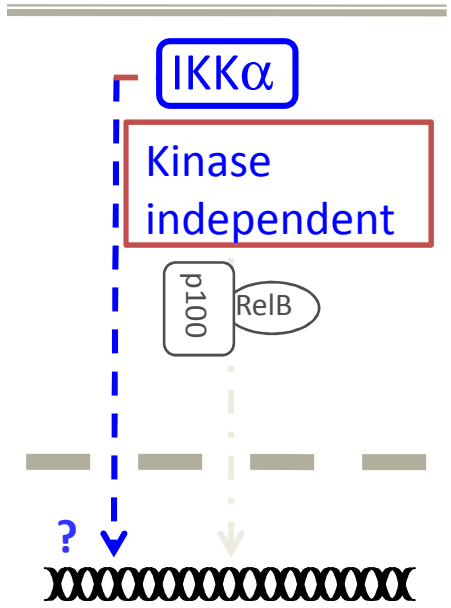


Olivotto, Otero et al., PLoS One 2013

IKKα KD leads to reduced collagenase activity by reducing the levels of MMP10, a collagenase activator



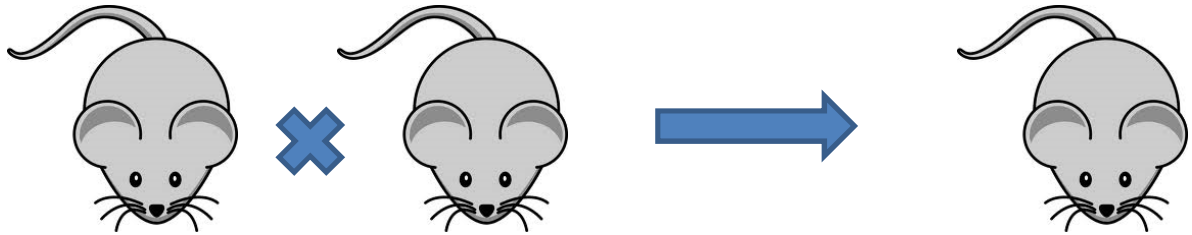




-Hypertrophic differentiation
-Mmp10 expression
–> collagenase activity

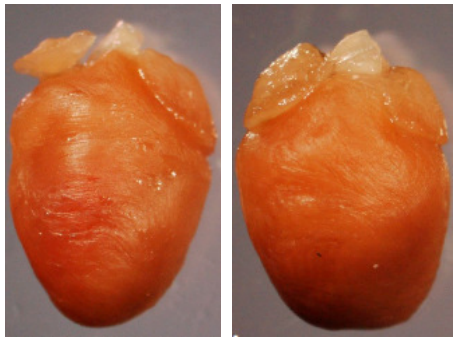
Olivotto, Otero et al. PLoS One 2013

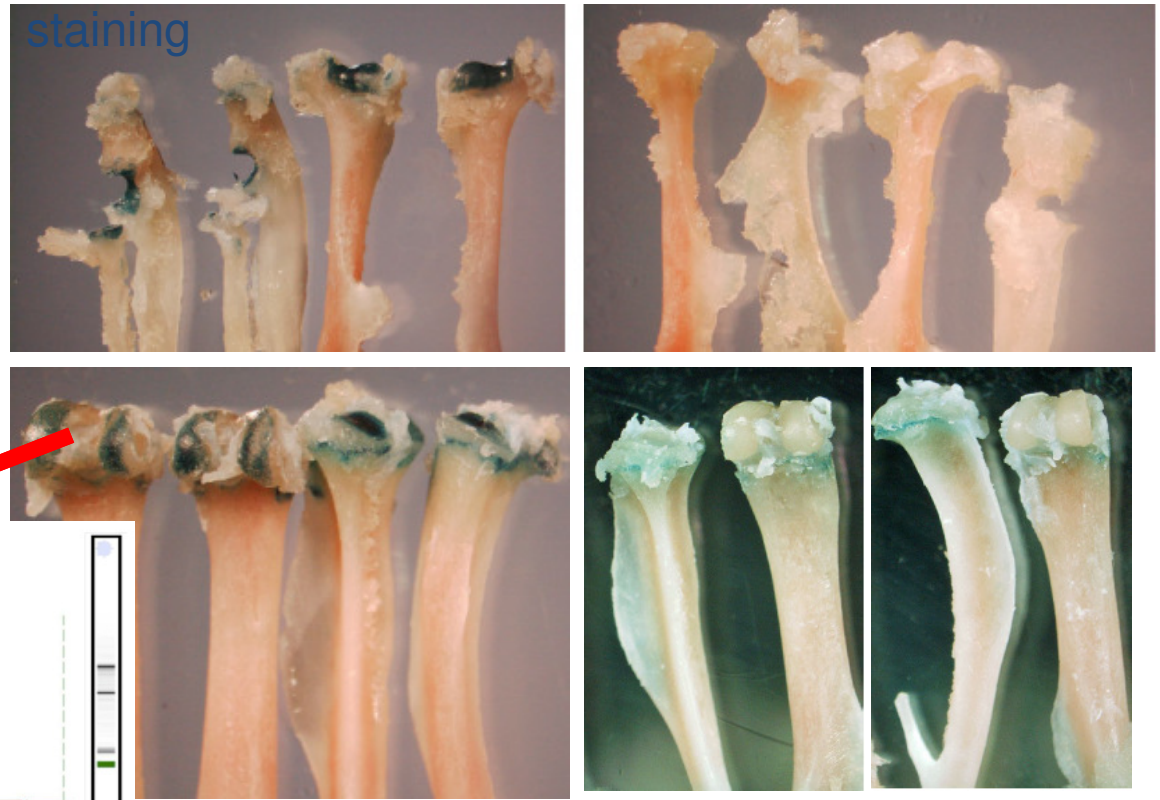
$IKK^{f/f}$;Agc1CreER$^{T2/+}$;$Lz^{f/f}$(R26R) mouse



$IKK^{f/f}$ Agc1-CreERT2 $IKK^{f/f}$;Agc1CreER$^{T2/+}$

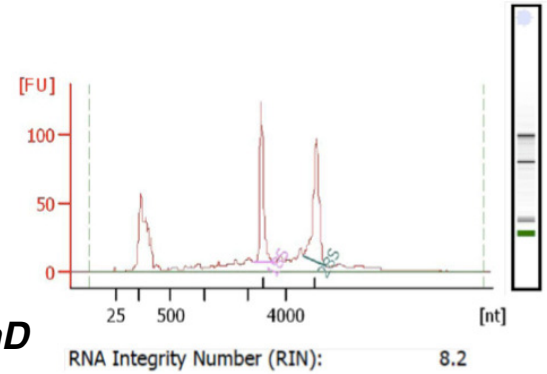
Verification of Cre-recombinase activity by X-gal staining





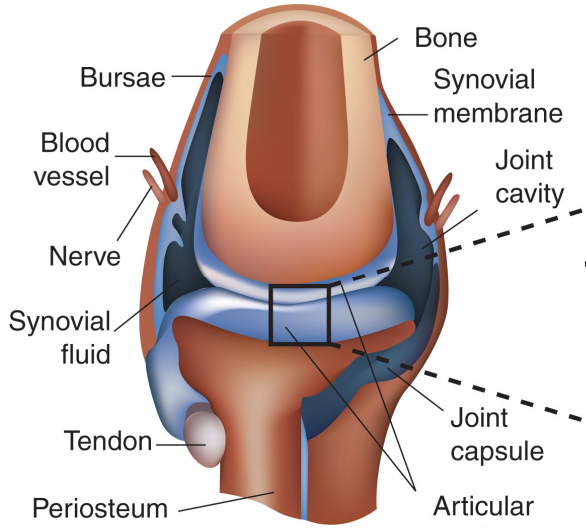
RNAseq and microRNA profiling

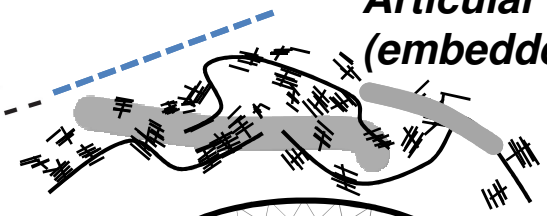
Kirsty Culley, PhD



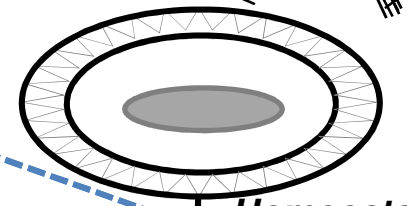
noxifen treated

Oil (vehicle)-treated





Articular chondrocyte (embedded in ECM)



Inflammatory signals from tissue environment, chronic biomechanical stress, and ageing

Homeostasis compromised

Epigenomic alterations

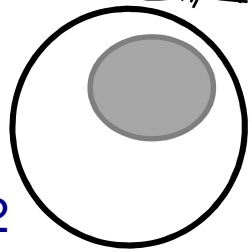
-HDAC 4 & 7 suppressed
-HDAC 1 & 2 up-regulated
MicroRNAs potentially suppressed
(-25*, -26a, -27a&b, -29a, -140, -146a, -149, -210, -337, -373)

-Gene specific CpG de-methylations
-SIRT1 levels reduced
MicroRNAs potentially up-regulated
(-9, -16, -22, -23b, -25*, -30b, -98, -103, -223, -377, -483, -509)

Degradative chondrocyte (early stage OA)

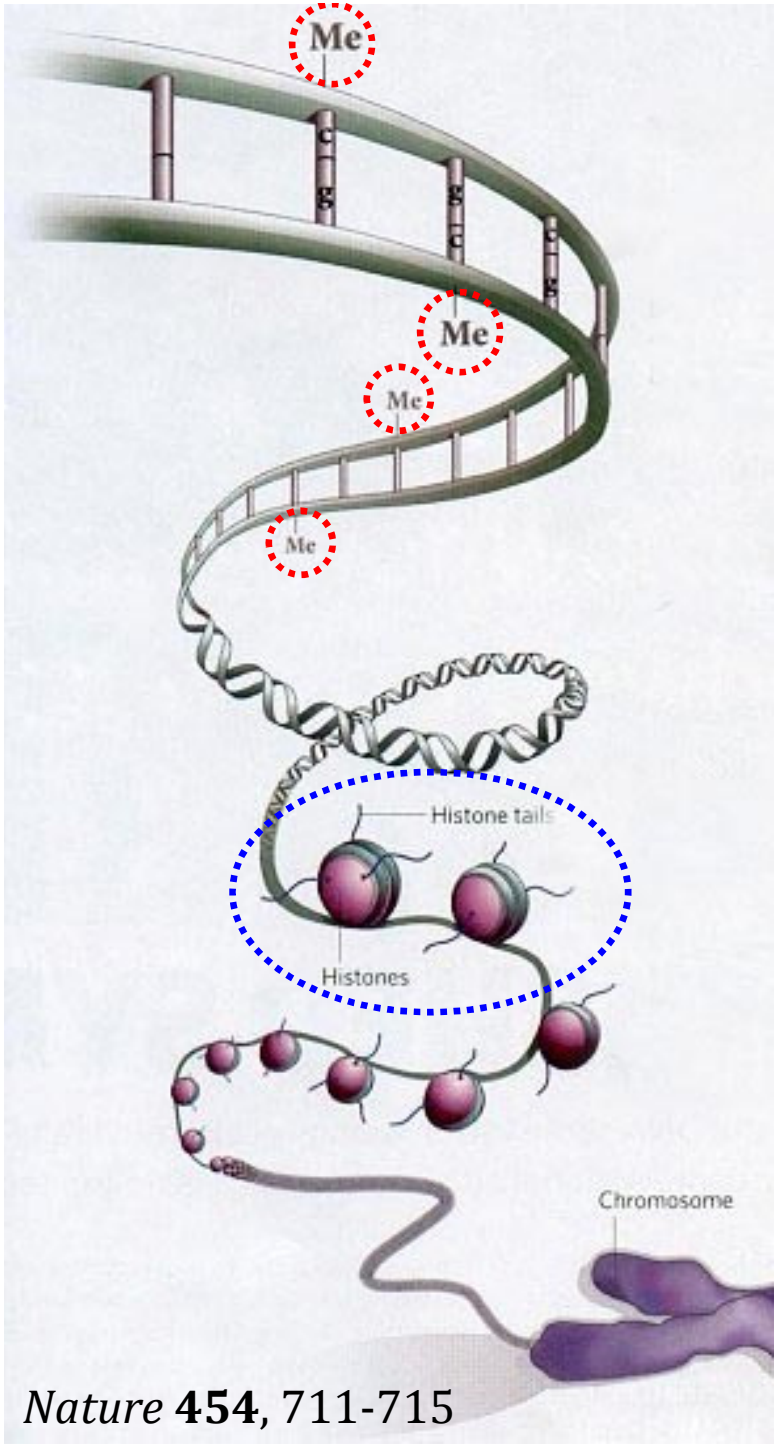
ECM degraded

Hypertrophic-like (late stage OA) chondrocyte



Apoptosis
Calcification
Osteophyte formation

Goldring & Marcu, Trends Mol Med 2012



Nature **454**, 711-715

Epigenetics: **heritable changes** in gene function that are not due to changes in DNA sequence

DNA methylation

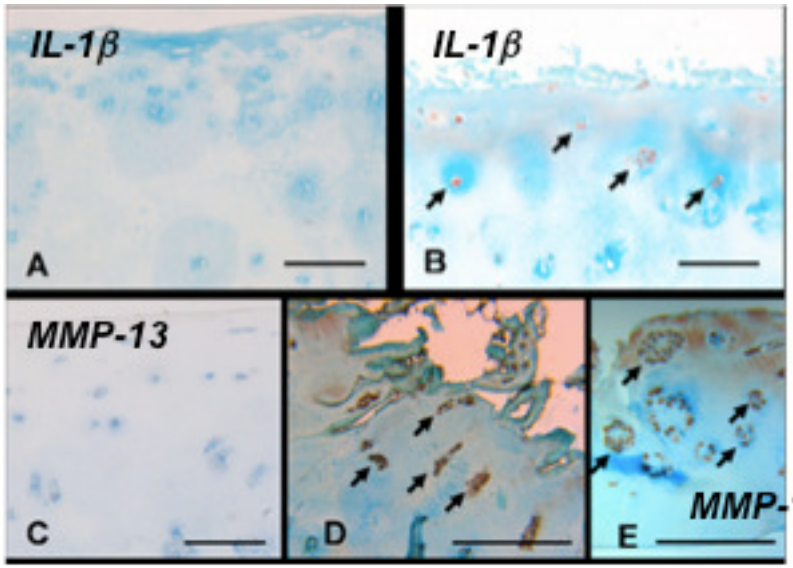
In promoter

↓ ↑

Histone modifications

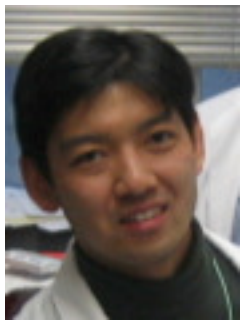
Acetylation
Methylation etc..

DNA de-methylation of CpG sites stimulates inflammatory and catabolic genes in OA cartilage

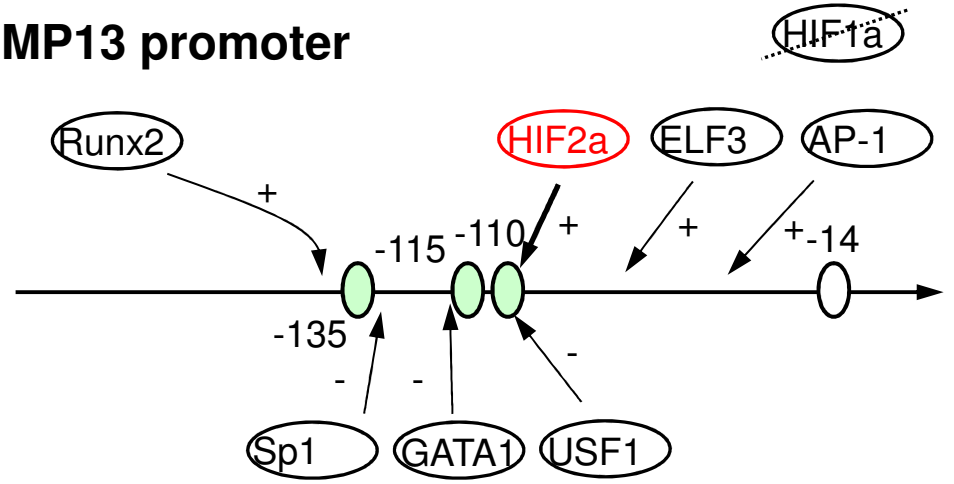


Roach et al. Arthritis Rheum 2005
Hashimoto et al., Arthritis Rheum 2009
Imagawa et al., BBRC 2010

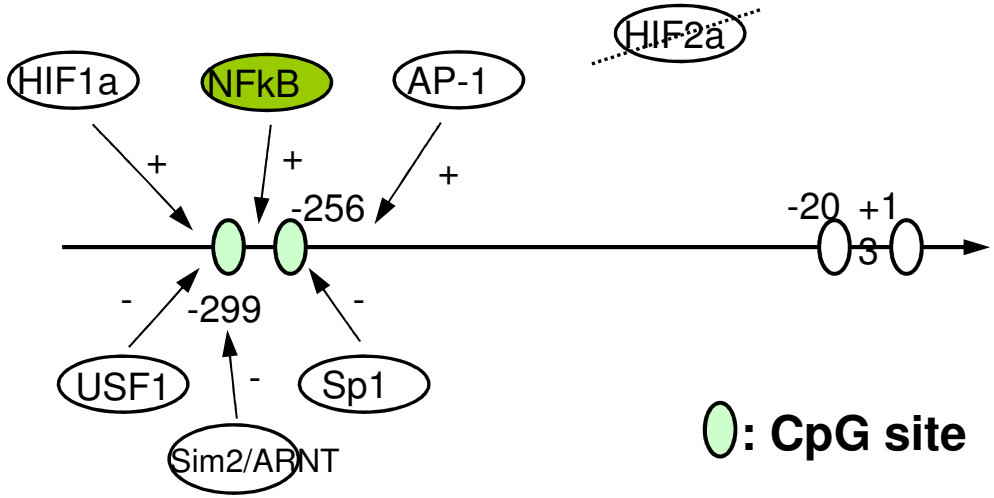
MMP-3, 9, 13
ADAMTS4
IL1B
NOS2 (deAndres)
COL9A1 (Imagawa)



MMP13 promoter



IL1B promoter



Hashimoto et al., J Biol Chem 2013

Transcriptional regulation of endochondral ossification by HIF-2α during skeletal growth and osteoarthritis development

Taku Saito[1,2], Atsushi Fukai[1], Akihiko Mabuchi[3], Toshiyuki Ikeda[2], Fumiko Yano[4], Shinsuke Ohba[4], Nao Nishida[3], Toru Akune[5], Noriko Yoshimura[5], Takumi Nakagawa[1], Kozo Nakamura[1], Katsushi Tokunaga[3], Ung-il Chung[4] & Hiroshi Kawaguchi[1]

Hypoxia-inducible factor-2α is a catabolic regulator of osteoarthritic cartilage destruction

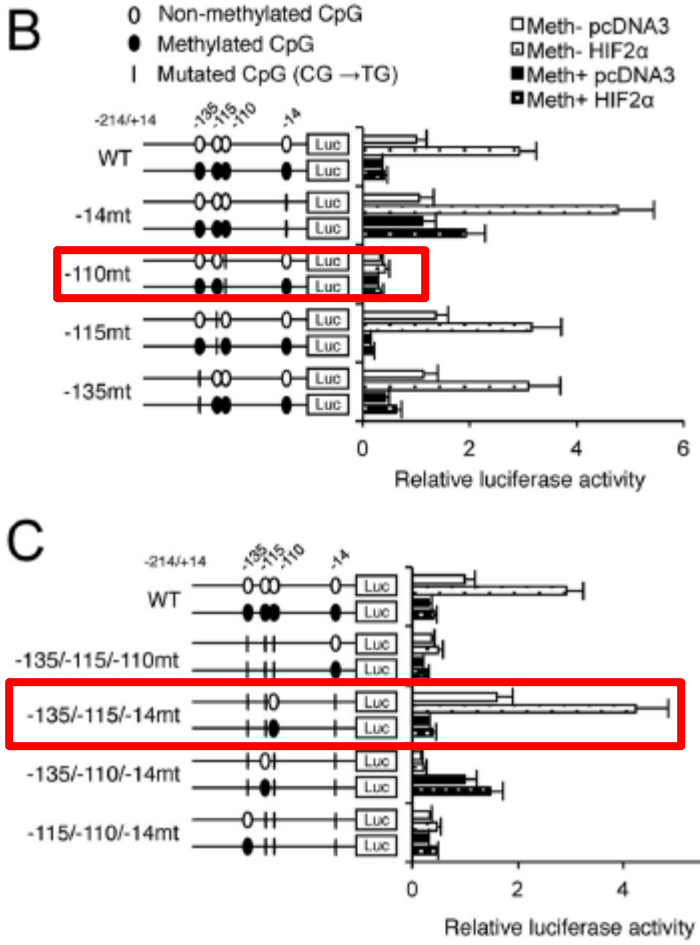
Siyoung Yang[1], Jonghwan Kim[1], Je-Hwang Ryu[1], Hwanhee Oh[1], Churl-Hong Chun[2], Byoung Ju Kim[3], Byoung Hyun Min[3] & Jang-Soo Chun[1]

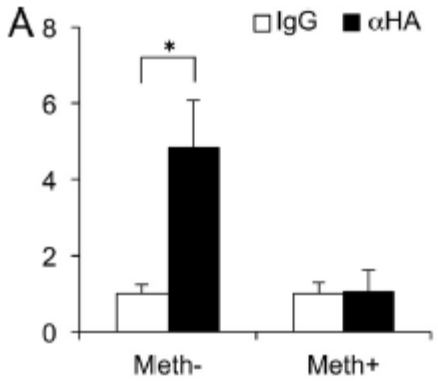
→ HIF-2α induces MMP-13 in a NF-κB-dependent manner

Regulated Transcription of Human Matrix Metalloproteinase 13 (*MMP13*) and Interleukin-1β (*IL1B*) Genes in Chondrocytes Depends on Methylation of Specific Proximal Promoter CpG Sites*

Received for publication, September 21, 2012, and in revised form, January 14, 2013 Published, JBC Papers in Press, February 15, 2013, DOI 10.1074/jbc.M112.421156

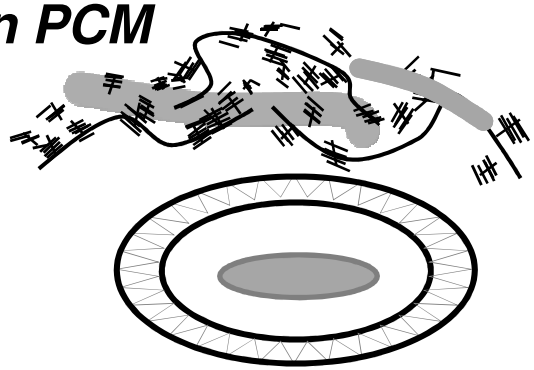
Ko Hashimoto[‡§], Miguel Otero[‡], Kei Imagawa[¶], María C. de Andrés[¶1], Jonathan M. Coico[‡], Helmtrud I. Roach[¶†], Richard O. C. Oreffo[¶], Kenneth B. Marcu[**‡‡], and Mary B. Goldring[‡2]





(A) CpG methylation attenuates HIF-2α binding to the *MMP13* promoter (ChIP assay) and (B,C) impairs HIF-2α-driven *MMP13* promoter transactivation

Resting articular chondrocyte embedded in PCM



Homeostasis
Anabolic factors
UPR/ER stress
Autophagy
Epigenetics, miRNA

Alk5 (Smad2/3)
>>Alk1 (Smad1/5/8)
HIF-1α (hypoxia)

OA Risk Factors

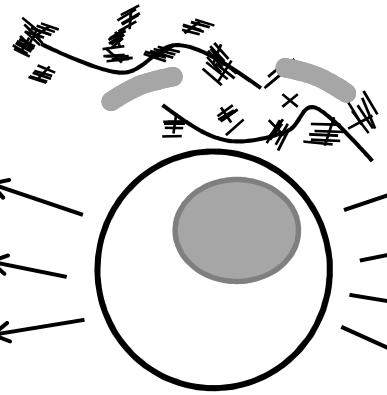
Inflammation
Mechanical stress
Oxidative stress
Ageing, etc.

Canonical NF-κB (IKKβ)
TLRs, ROS
Etc.

Release from growth arrest & phenotypic modulation

Alk1 (Smad1/5/8) >>Alk5
TGFβR2/MCP-5 (***A. Spagnoli***)
HIF-2α & Zinc-ZIP8-MTF(***J-S Chun***)
Noncanonical NF-κB (IKKα) (***M. Otero***)
FOXA2 (***A. Ionescu***)

Dual anti-IL-1α/β (***RV Kamath***)



IL-1, other cytokines
Chemokines
MMPs, ADAMTS

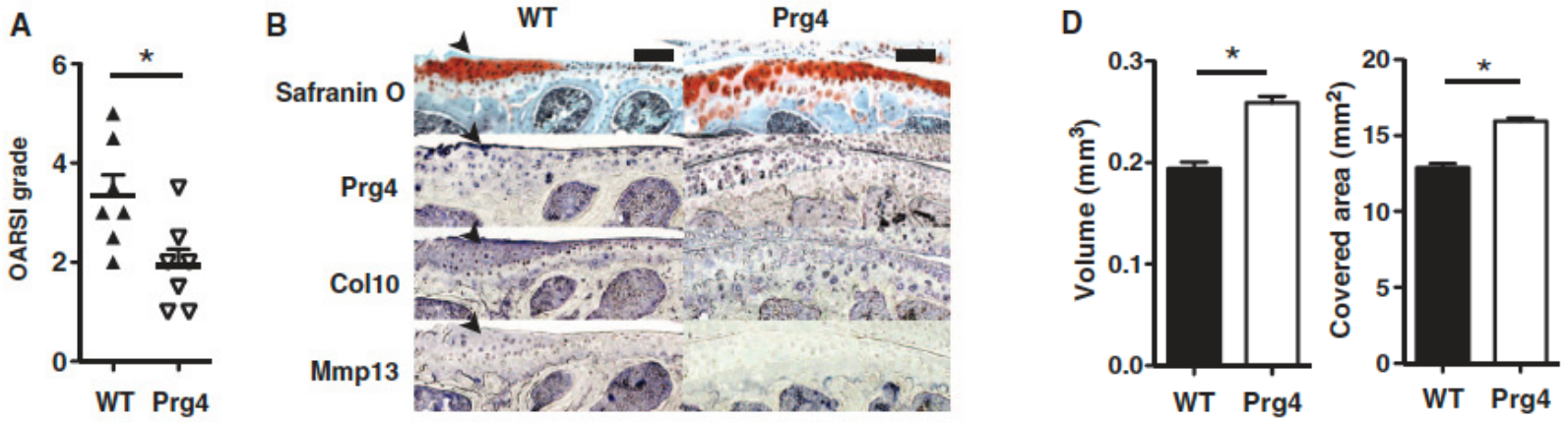
Kartogenin/Runx1 (***K. Johnson***)

Runx2, COL10
MMP13/MMP10
Calcification
Osteophyte formation

M. Goldring: Cartilage GRC 2015

PRG4 expression protects against the development of OA

- **Generated *Prg4* transgenic mice under the cartilage-specific type II collagen promoter**
  Mice partially protected from age-related OA cartilage pathology
  - **Lower OARSI OA grade compared to WT litter mates**
  - **No increase in *Col 10a1* or *MMP-13* expression**
  - **Preservation of cartilage volume and bone surface area covered by cartilage**



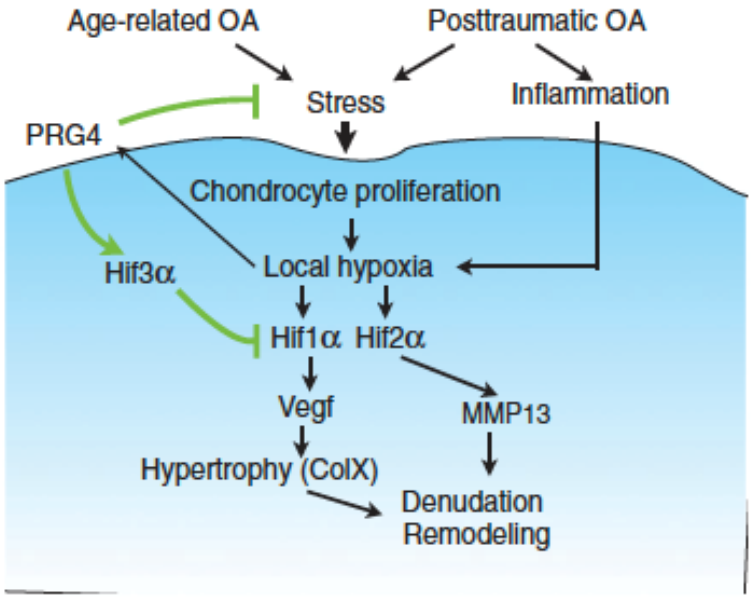
•*Prg4* transgenic mice showed minimal evidence of OA cartilage pathology after anterior cruciate ligament transection and protection from pain-related behavior

Ruan et al. Sci Transl Med. 2013 Mar 13;5(176):176ra34

PRG4 expression protects against the development of OA

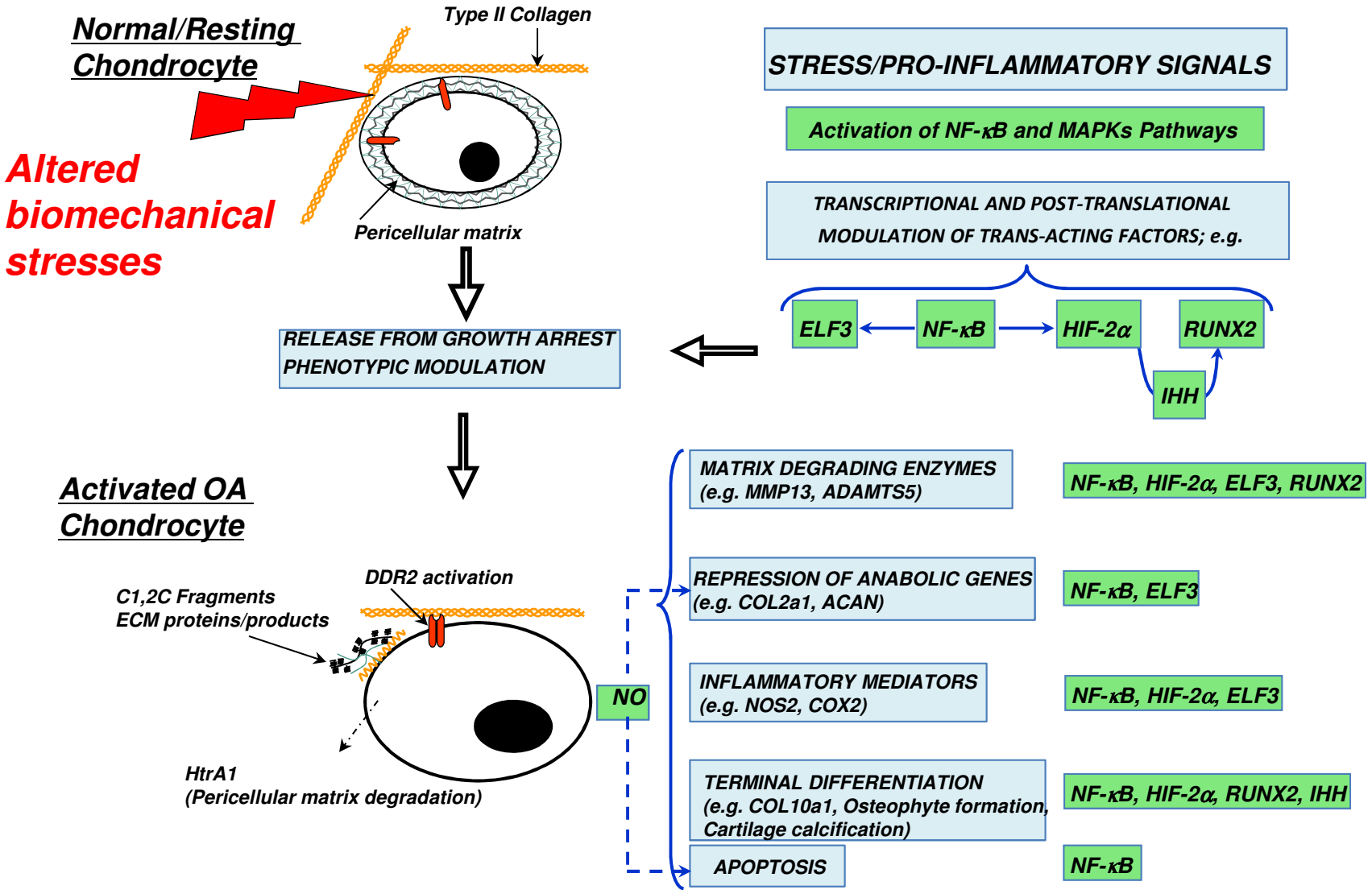
Ruan (Brendan Lee) et al., Sci Transl Med. 2013 Mar 13;5(176):176ra34

- Transcriptional profiling and pathway analysis of superficial chondrocytes after gene transfer revealed PRG4 overexpression prevented the induction of catabolic and anti-anabolic genes and pathways; mediated in part by up-regulation of HIF3α, which inhibits the adverse effects of HIF1α and 2α on chondrocytes

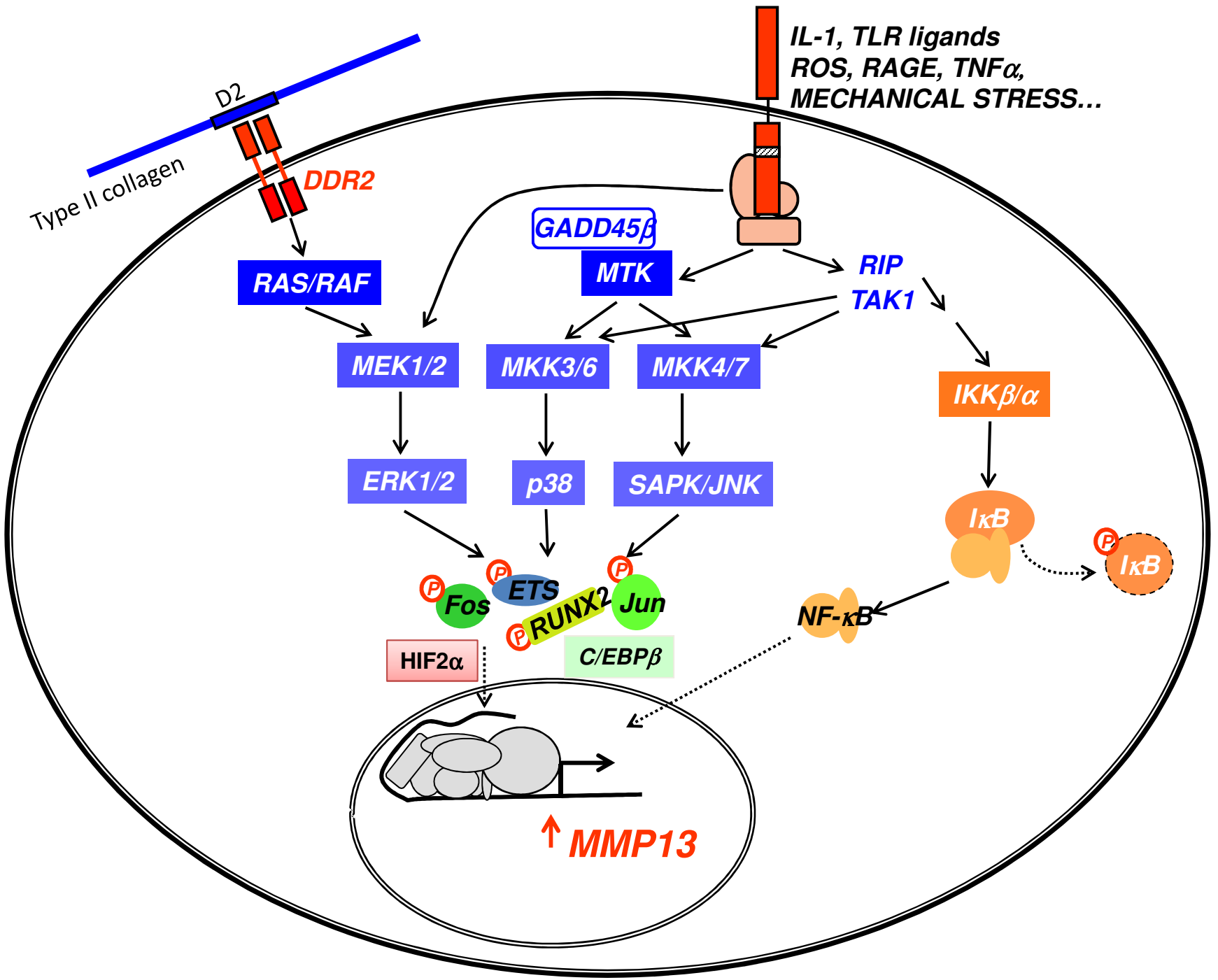


<u>Commentary</u>: These studies provide insights into the mechanism of action of lubricin in maintaining cartilage homeostasis and identify lubricin as a potential therapeutic intervention to slow the progression of OA pathology.

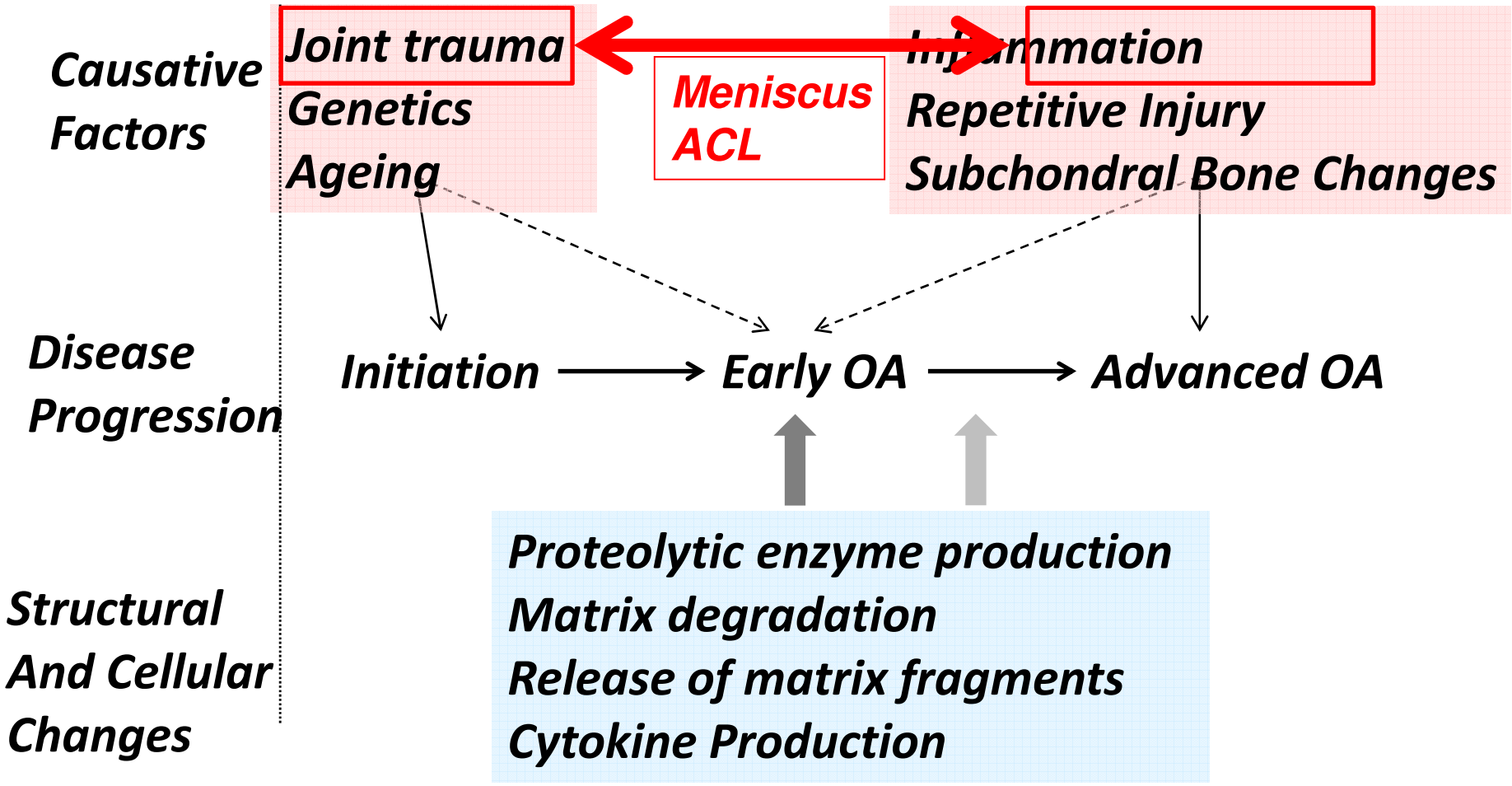
Phenotypic modulation in response to stress/inflammatory signals: Key role of NF-κB



Miguel Otero: Adapted from Goldring, Marcu, et al., ECM 2011


IL-1, TLR ligands
ROS, RAGE, TNFα,
MECHANICAL STRESS…
D2
Type II collagen
DDR2
GADD45β
MTK
RIP
TAK1
RAS/RAF
MEK1/2
MKK3/6
MKK4/7
IKKβ/α
ERK1/2
p38
SAPK/JNK
IκB
P
IκB
P
Fos
P
ETS
P
RUNX2
Jun
NF-κB
HIF2α
C/EBPβ
MMP13

Heterogeneity of OA



Jenny Scott: Modified from Goldring & Goldring: J Cell Physiol, 2007

Laboratory of Cartilage Biology

MMP-13

Elf3

RNAseq
miRNA

Histology
Surgery

Kirsty Culley

Justin Quinn

Jun Chang

Elisabeth Wondimu

Miguel Otero

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R01-AG022021
R21-AR054887
RC4-AR060546

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