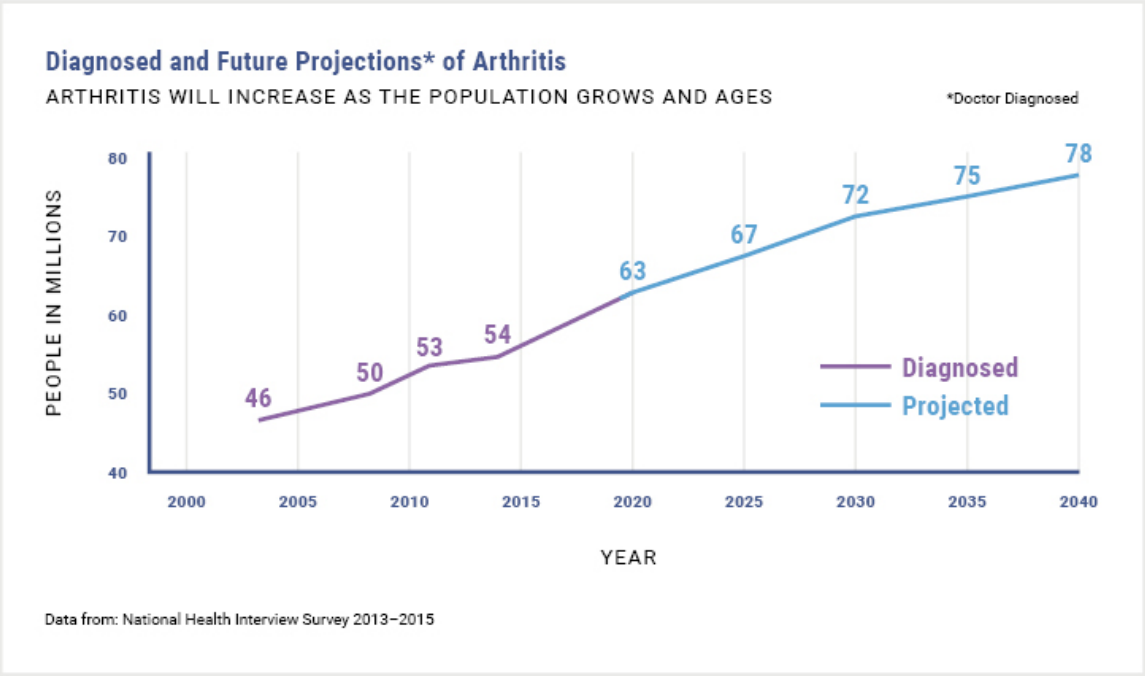
Overcoming arthritis:

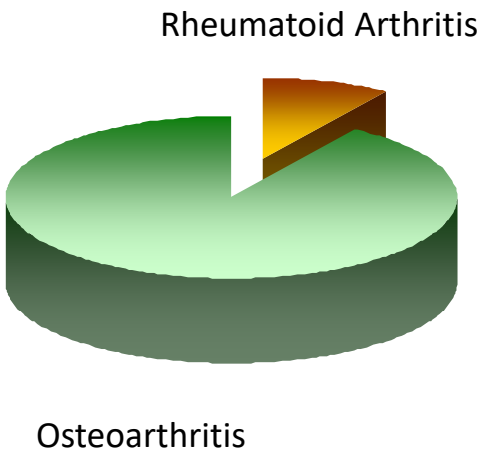
Science offers hope for removing achy joints from the aging equation

Martin Lotz, MD

Scripps Research
Science Changing Life

Aging and Arthritis

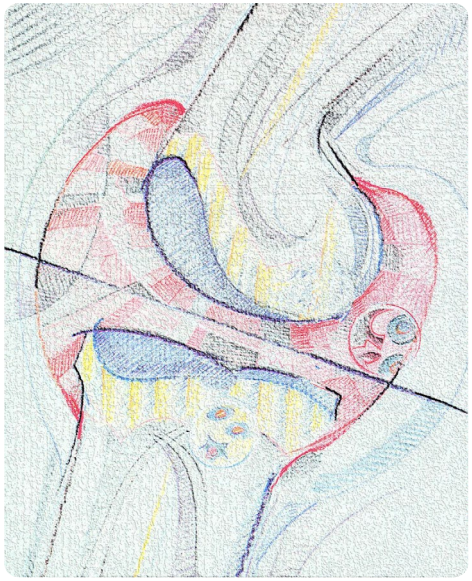


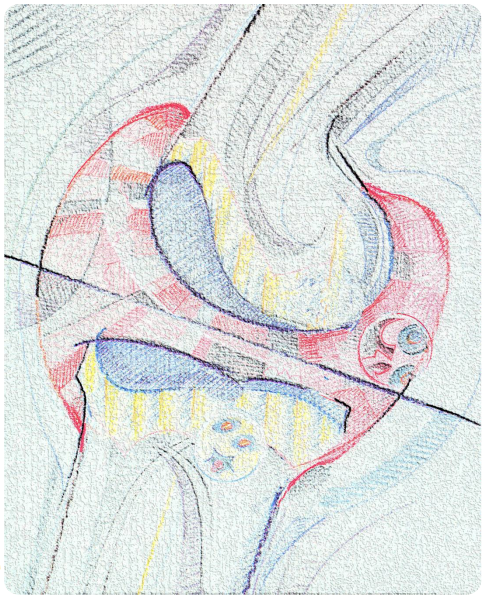


More than 60 million Americans are affected by arthritis.

More than half of individuals with symptomatic knee osteoarthritis are younger than 65.

More than 1 million total joint replacements per year.
Expected to increase to 4 million by 2030.





Osteoarthritis

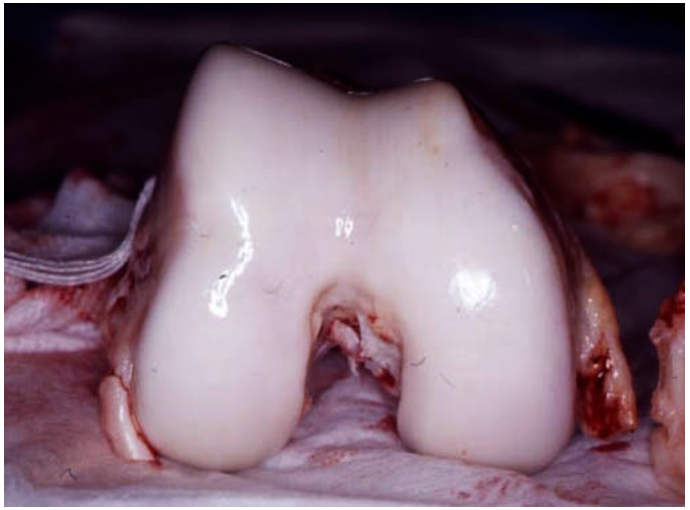
- **Disease impact and mechanisms of disease**
- Drug target discovery
- FoxO transcription factors for Osteoarthritis therapy
- Mohawk transcription factor for meniscus healing and Osteoarthritis prevention

Osteoarthritis is a Serious Disease*

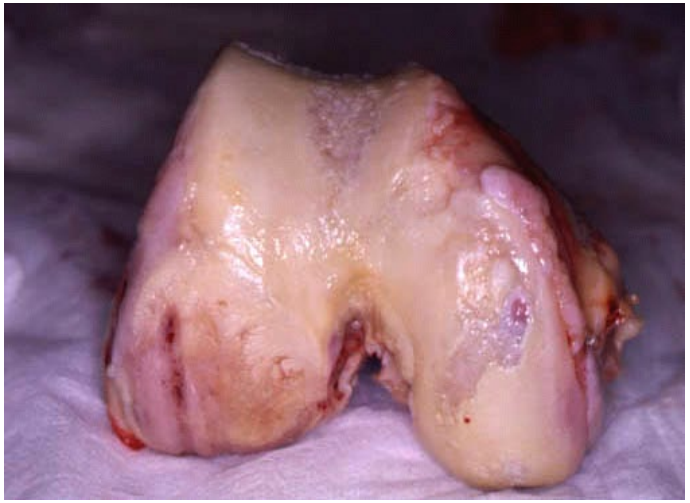
- Leading cause of pain
- Affects sleep quality, mood, and participation in every-day life
- 3rd most rapidly rising condition associated with disability, just being diabetes and dementia
- Limits person's ability to self-manage other conditions such as hypertension or diabetes
- Increases risk for developing heart disease by 50%
- Reduced levels of physical activity, comorbid conditions and adverse effects of medications lead to a **55% increase in all cause mortality**

*FDA 2018

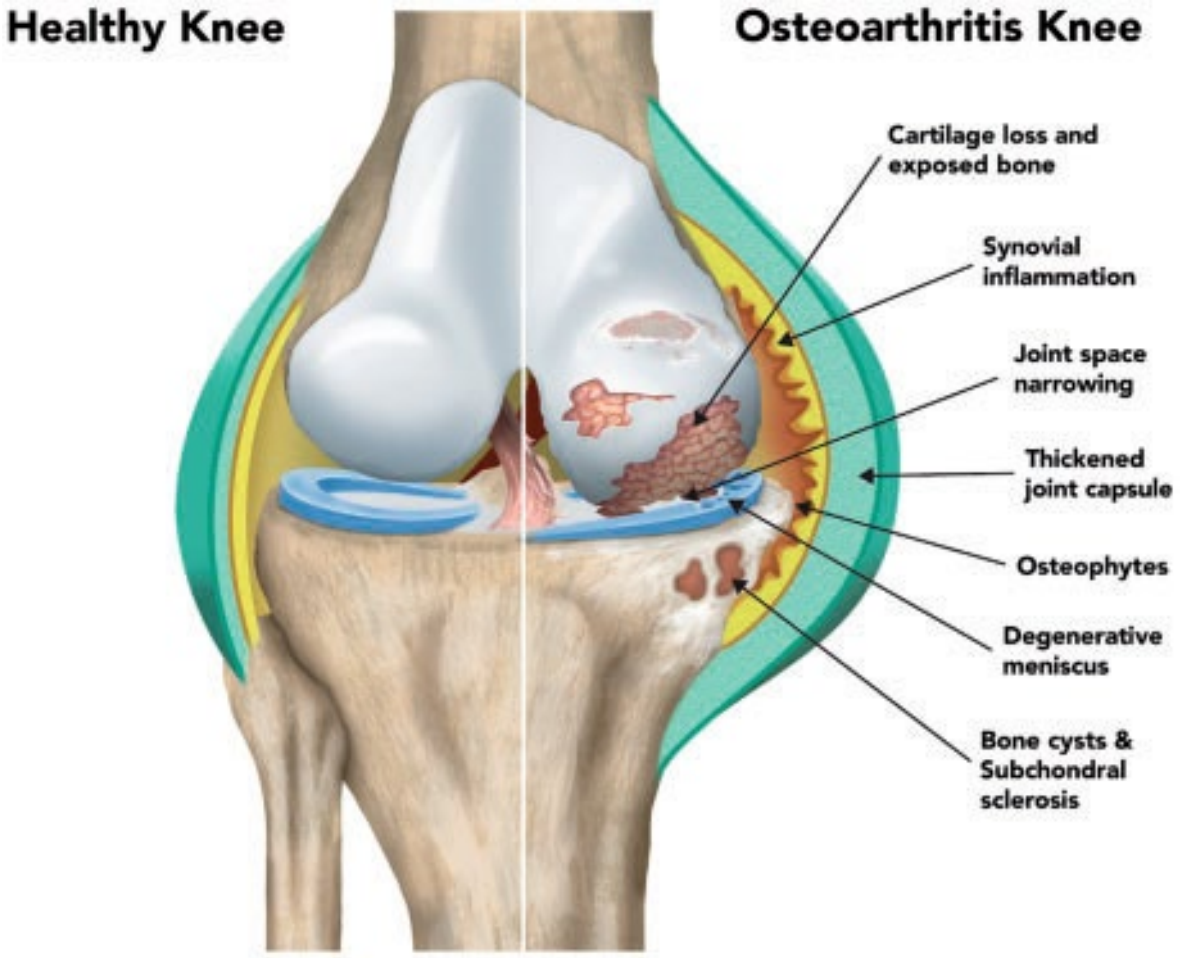
Osteoarthritis affects all joint tissues



Normal 24-year -old

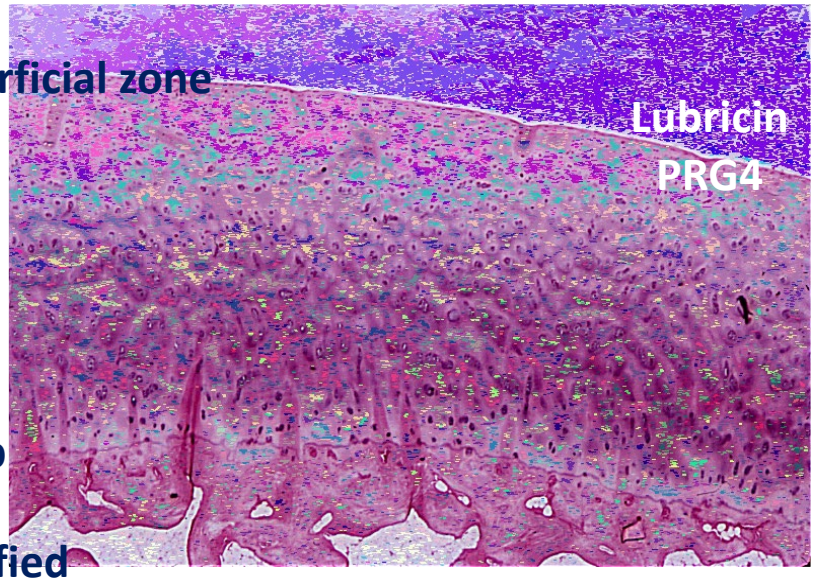


OA 64-year-old



Uhalte et al, *Hum Mol Genet*, 2017

Joint cartilage and biomechanical function



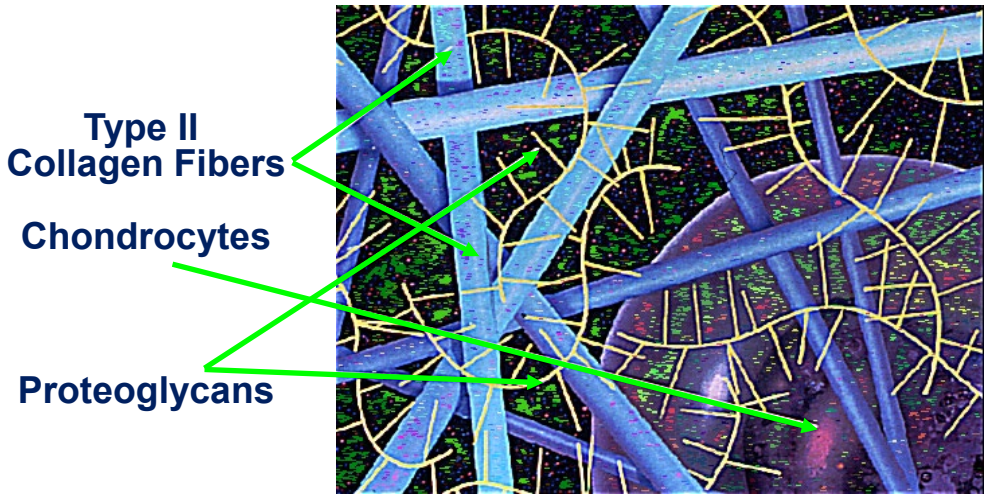
Collagen framework with proteoglycans

Collagen type II
- Structure
- Tensile strength

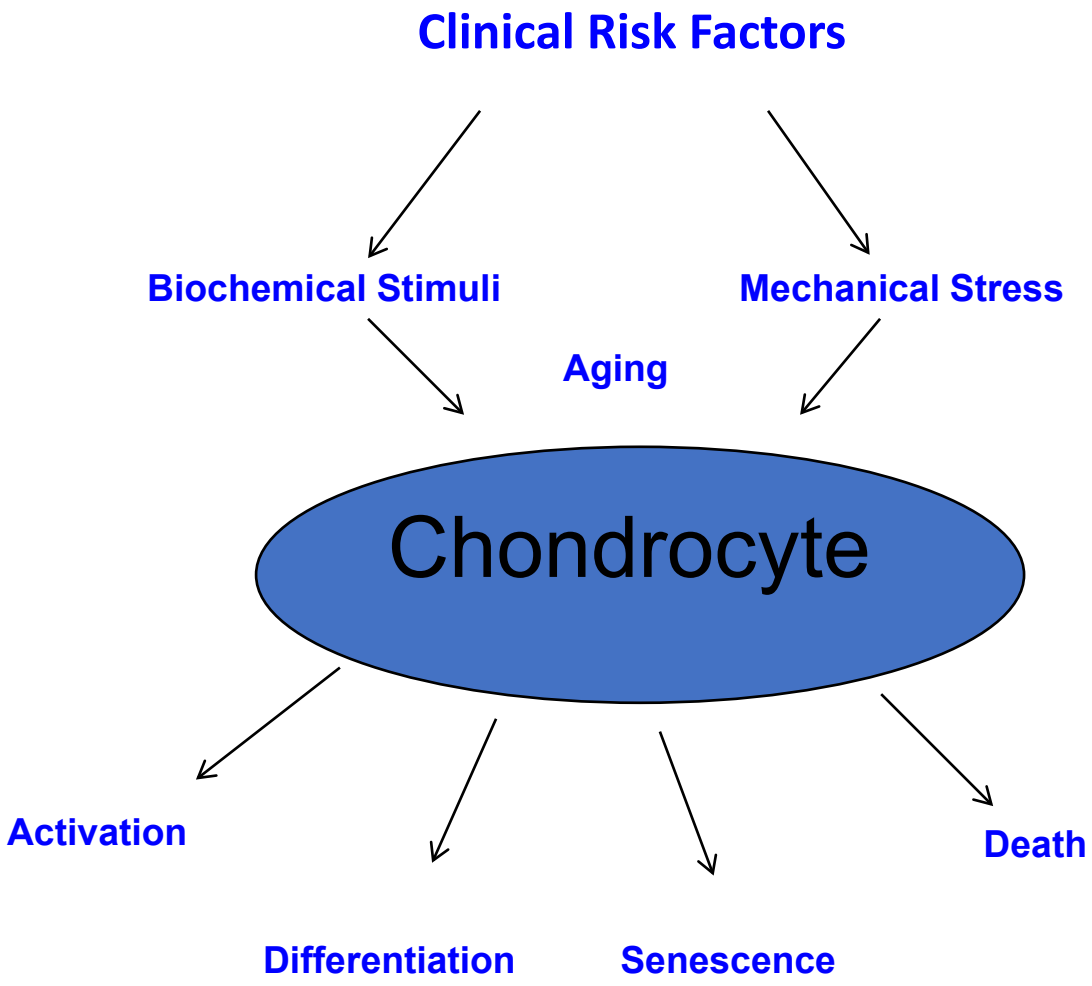
Aggrecan
- Core protein
- Glycosaminoglycan side chains
- Water binding: swelling pressure

Cartilage cells:
Chondrocytes
Progenitor cells

No nerve fibers
No blood vessels

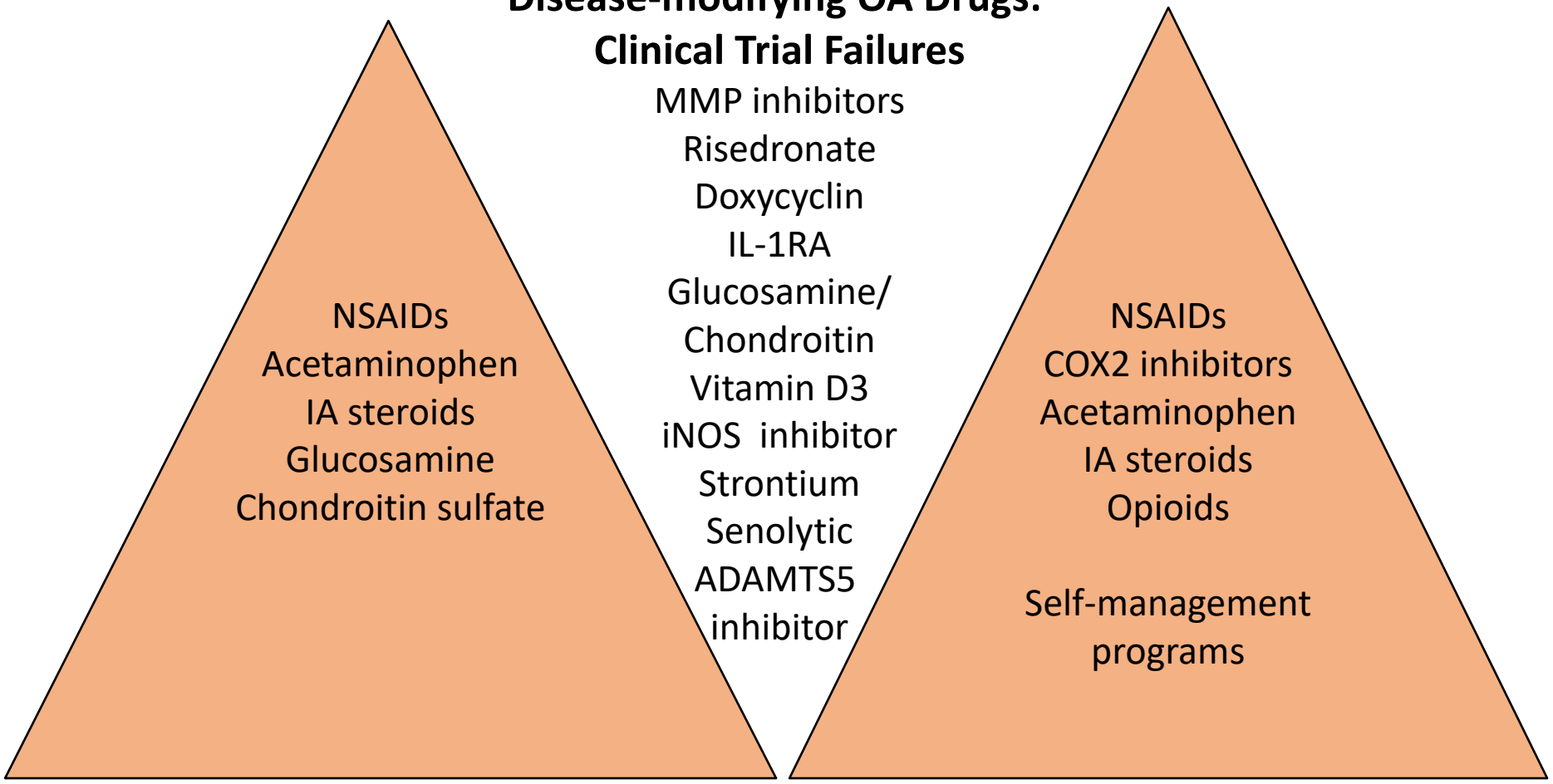


Pathways of cartilage destruction



Mediators of extracellular matrix destruction and inflammation

Osteoarthritis treatment



Treatment guidelines 1985

Treatment guidelines 2021

Potential reasons for failures of DMOAD clinical trials

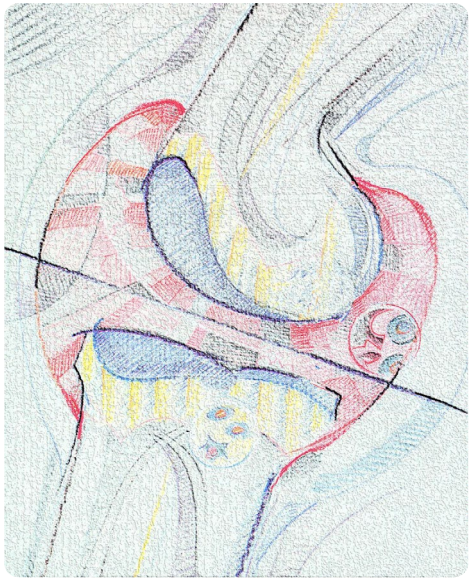
Disease-modifying OA drugs (DMOAD) that slow, or halt radiographic structural disease progression are not available

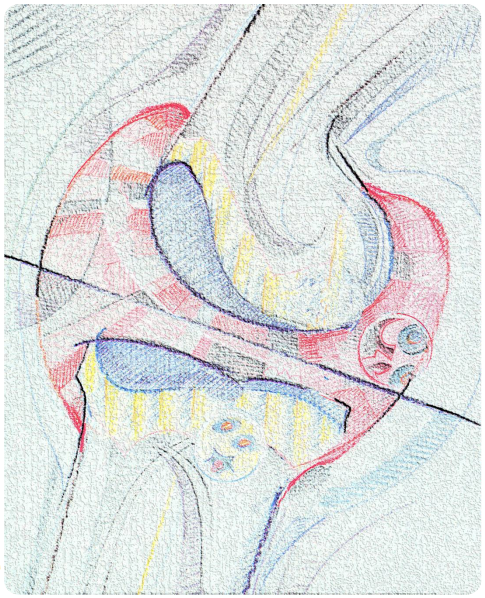
- **Clinical trials design**
  - Patient heterogeneity
  - Advanced stages of disease: calcification, amyloid
  - Burden of clinical risk factors (aging, obesity, overuse, injury)

- **Drug targets**
  - Large number of effector molecules in tissue destruction
  - Spectrum of inflammatory mediators
  - Diverse disease pathways

➢ **Need for better understanding of disease mechanisms and identification key drivers of tissue damage and pain**

➢ **Better drug targets**





Osteoarthritis

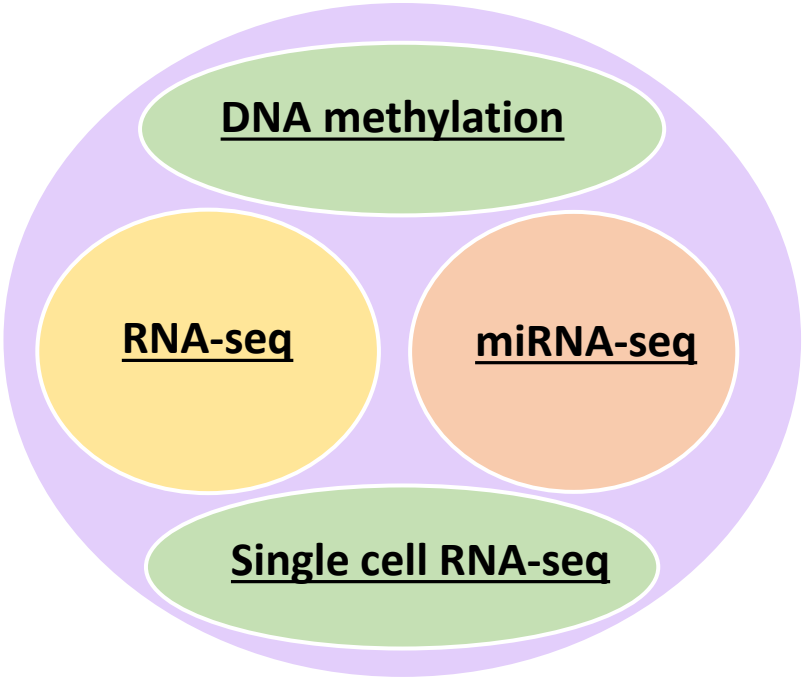
- Disease impact and mechanisms of disease
- **Drug target discovery using Omics analyses**
- FoxO transcription factors for Osteoarthritis therapy
- Mohawk transcription factor for meniscus healing and Osteoarthritis prevention

Joint Omics Analysis

Objectives

Characterize the transcriptomic and epigenomic landscapes of normal and OA articular cartilage and meniscus to identify genes and pathways that are central to OA pathophysiology and

prioritize potential therapeutic targets.



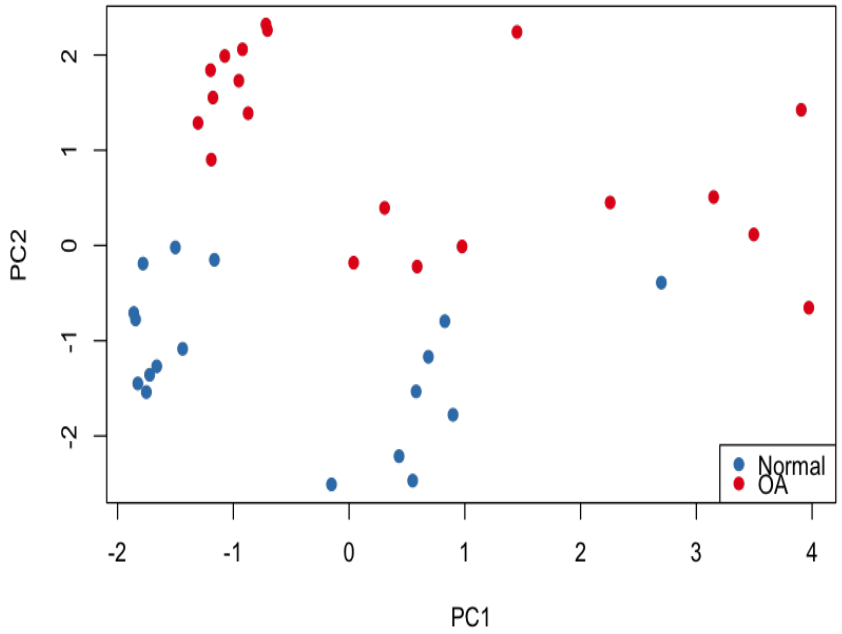
Focus on Transcription Factors

Rationale

Transcription factors are critical determinants of tissue identity by regulating expression of tissue specific genes.

Dysregulated transcription factors control expression of disease promoting genes and are promising therapeutic targets.

RNA-sequencing analysis of normal vs. Osteoarthritis human cartilage

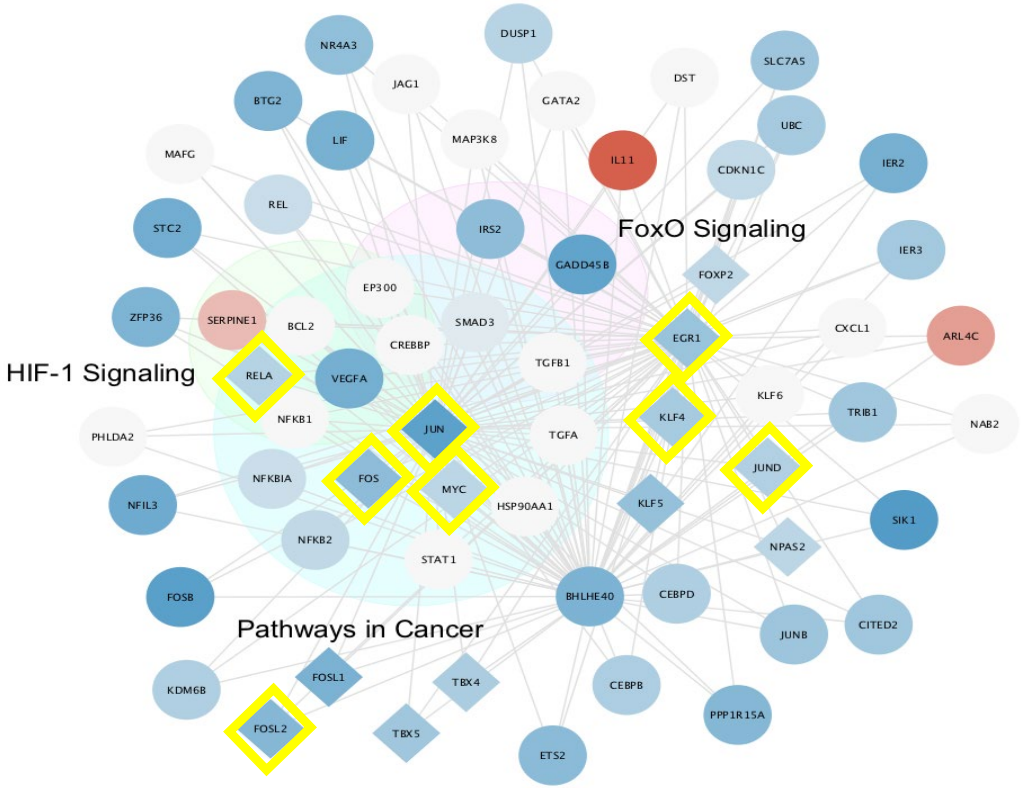


- 18 normal and 20 OA samples
- 12,467 genes considered expressed
- 1332 Differentially expressed genes
- 630 upregulated, 702 downregulated in OA

Fisch et al. Osteoarthritis Cartilage. 2018

Dysregulated transcription factors in Osteoarthritis

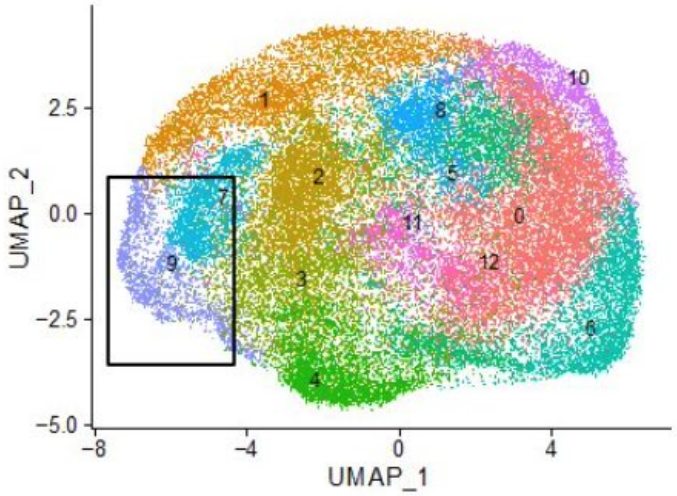
12,467 cartilage expressed genes → 1332 Differentially expressed genes → 93 transcription factors are differentially expressed in OA → 73/93 **suppressed** → Transcription factors targeting differentially expressed genes

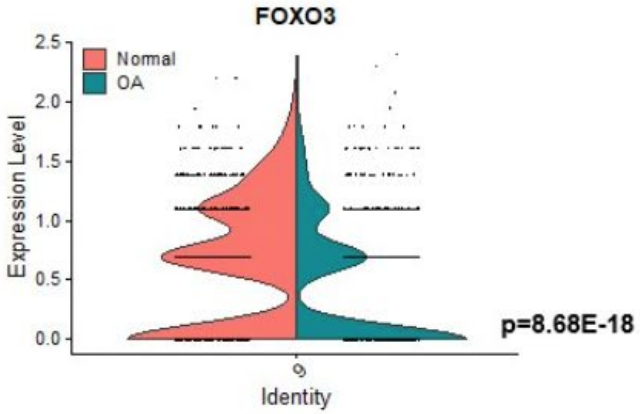


Fisch et al. Osteoarthritis Cart. 2018

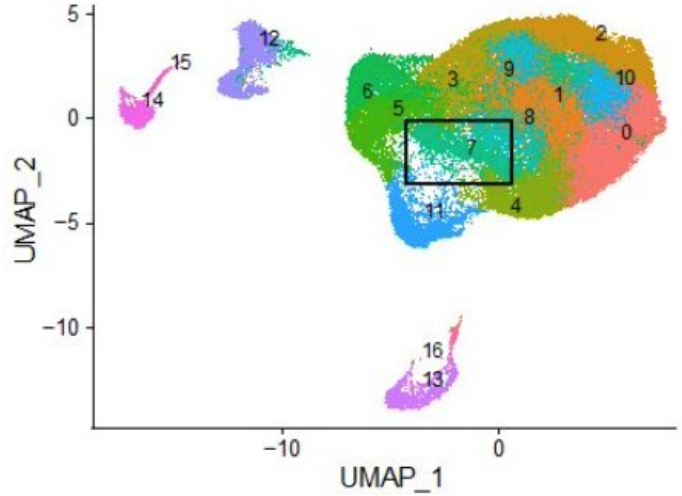
FoxO suppression in pathogenic cell subsets

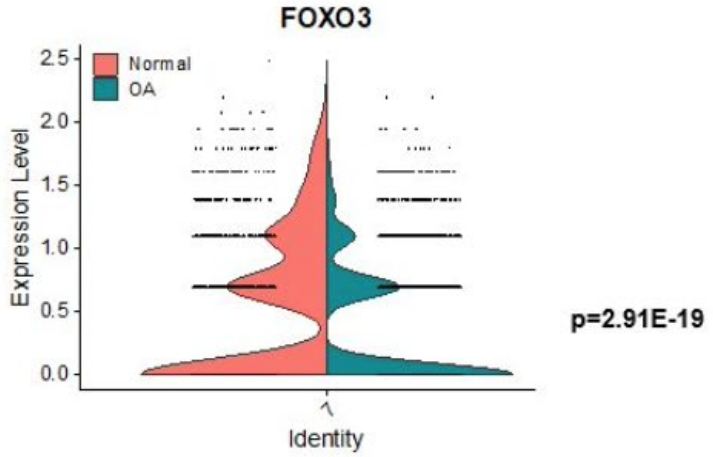
Cartilage
Normal n=3
OA n=3





Meniscus
Normal n=4
OA n=4





Hannah Swahn in preparation

Summary of RNA-seq study

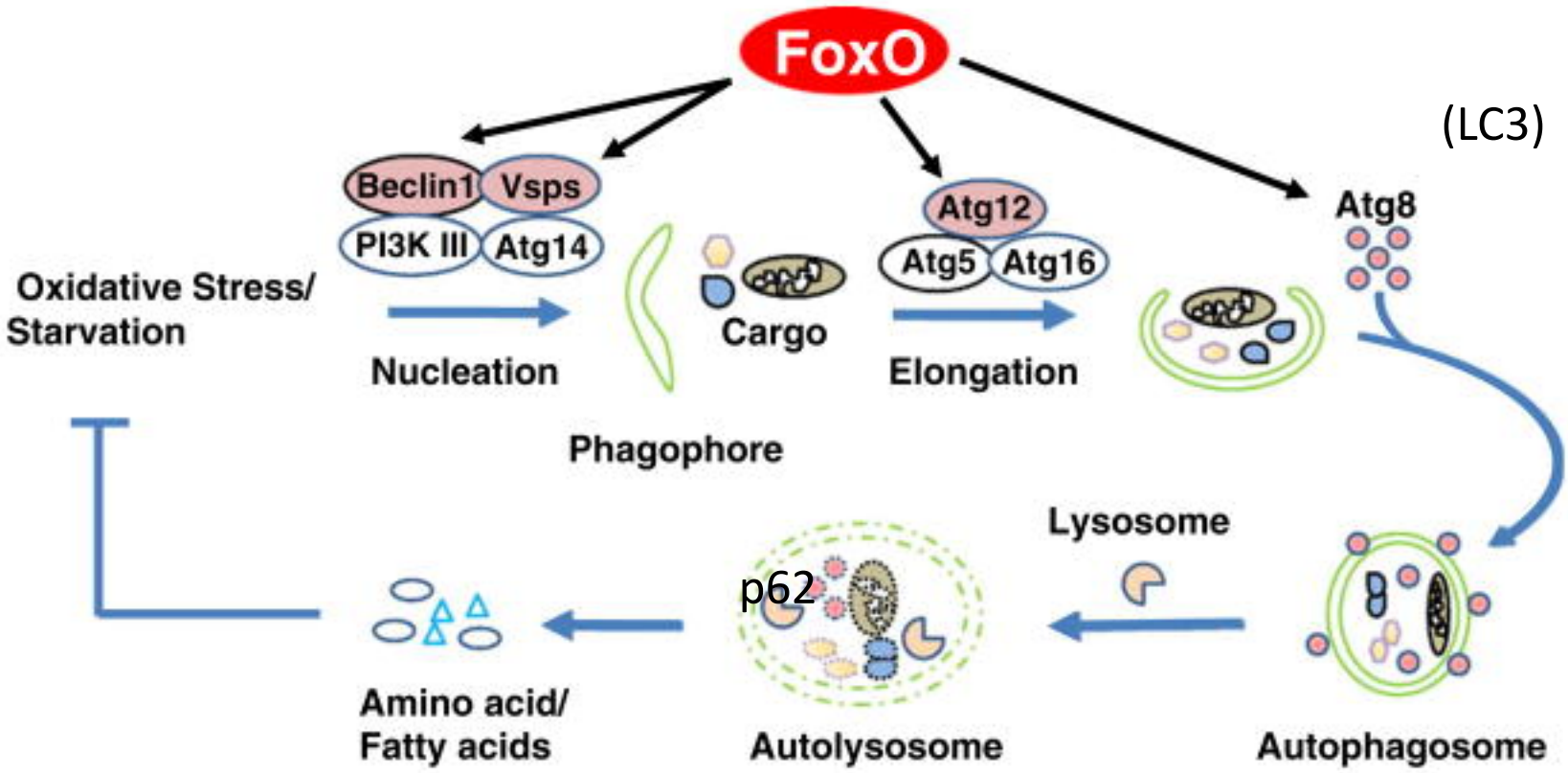
- OA cartilage has high expression of genes involved in ECM remodeling and reduced expression of genes involved in tissue homeostasis (HIF-1, **FoxO**, circadian rhythm).

- **Deficiency of FoxO transcription factors is a mechanism for disease-promoting gene expression patterns in OA cartilage**.

Osteoarthritis

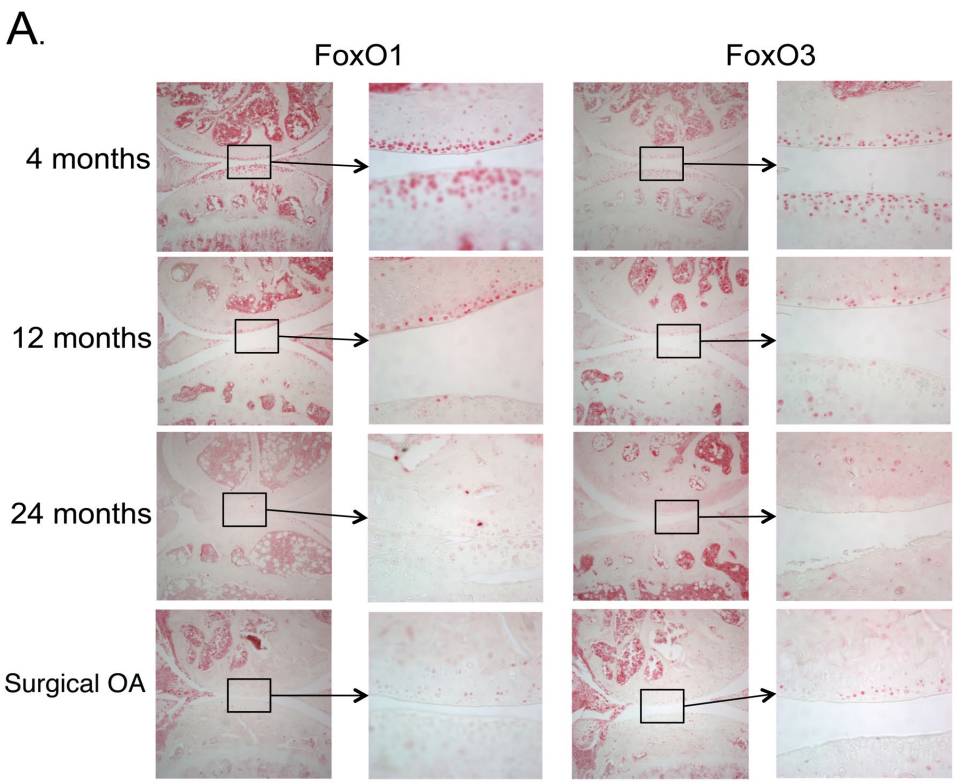
- Disease impact and mechanisms of disease
- Drug target discovery using Omics analyses
- **FoxO transcription factors for Osteoarthritis therapy**
- Mohawk transcription factor for meniscus healing and Osteoarthritis prevention

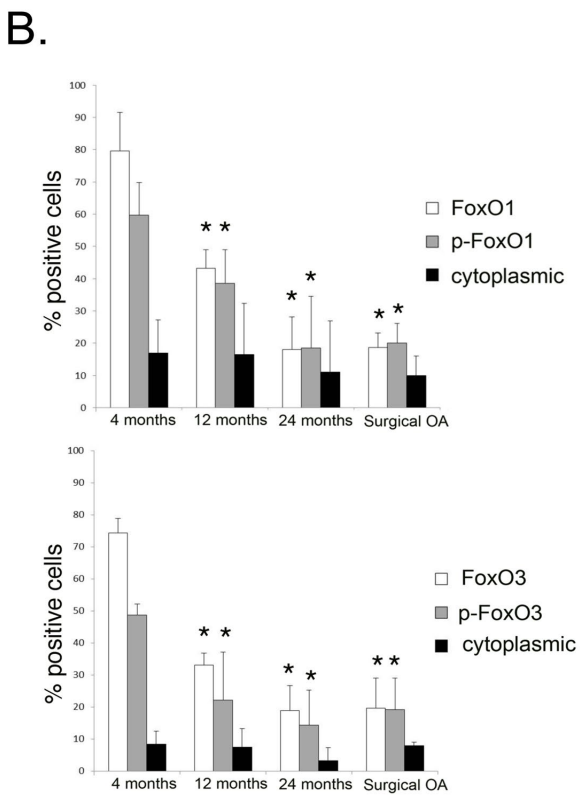
Forkhead box O (FoxO) transcription factors and autophagy



FoxO play a crucial role in regulation of oxidative stress resistance to promote longevity and reduce age-related disease.

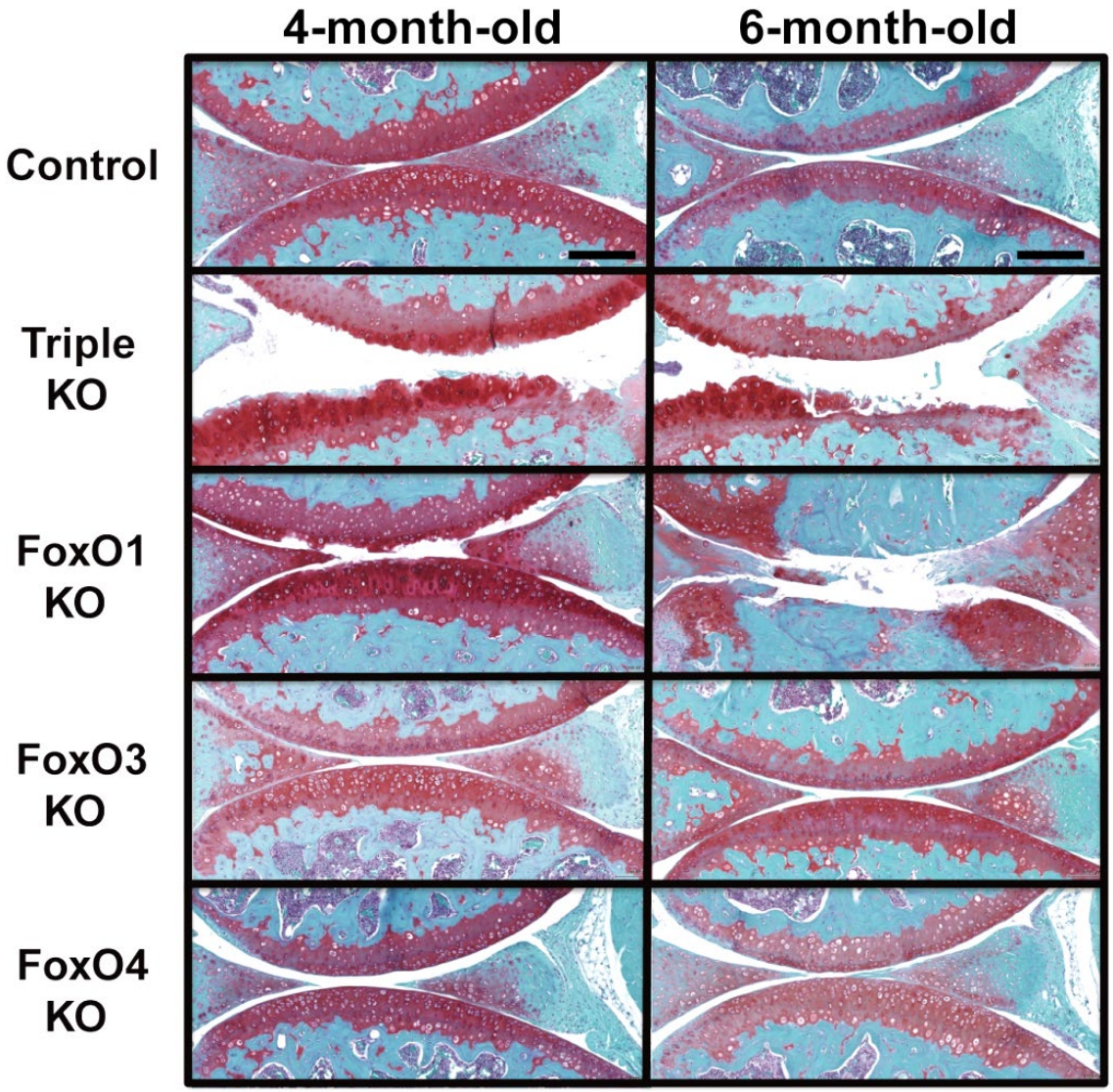
FoxO expression in osteoarthritis mouse joints

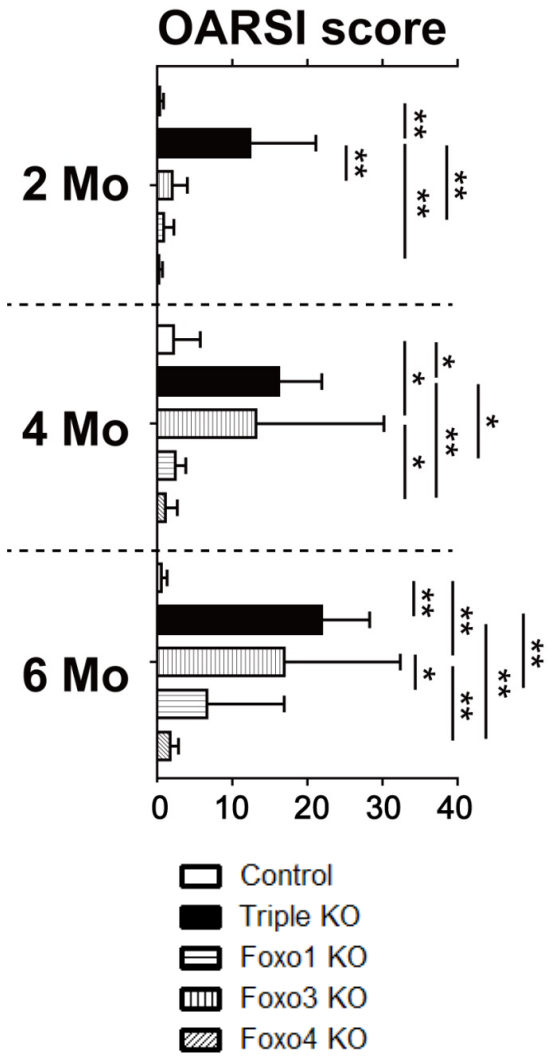




Akasaki et al. Arthritis Rheumatol. 2014

Spontaneous cartilage degradation in Col2CreFoxO triple KO and Col2CreFoxO1 KO mice





Matsuzaki et al. Sci Transl Med. 2018

Postnatal FoxO deletion

Control  FoxO1/3/4$^{Lox/lox}$;Aggrecan-Cre $^{ERT-}$

Triple KO  FoxO1/3/4$^{Lox/lox}$;Aggrecan-Cre $^{ERT+}$

Intraperitoneal Tamoxifen injection

RNA collection

Spontaneous OA model

Day0 1 2 3 4

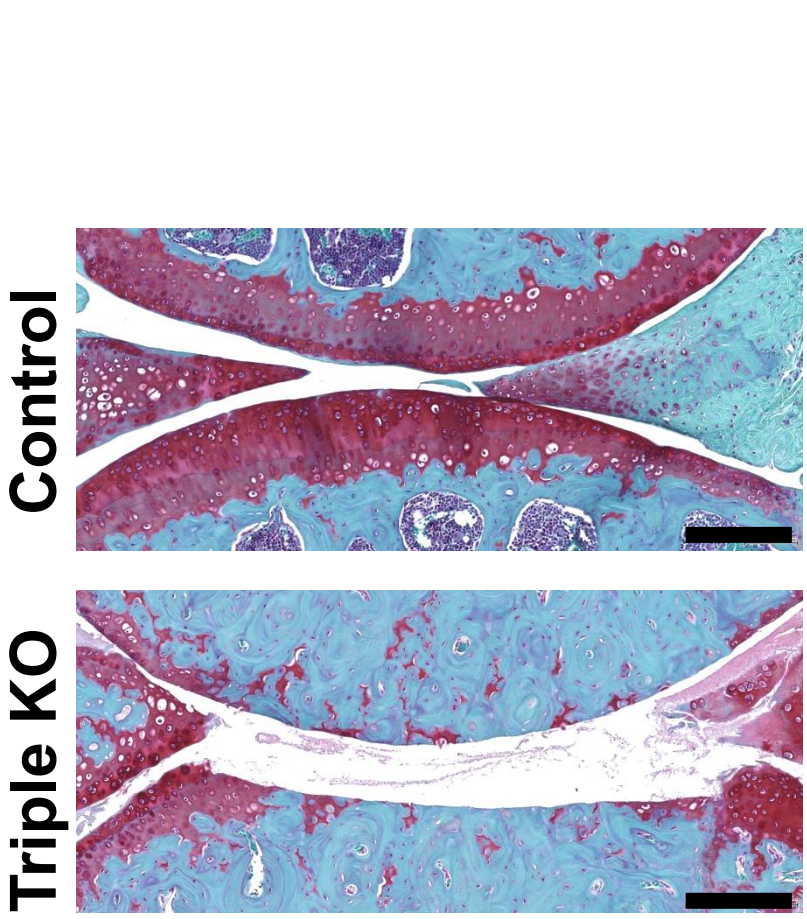
2W **3W** **2M** **5M**

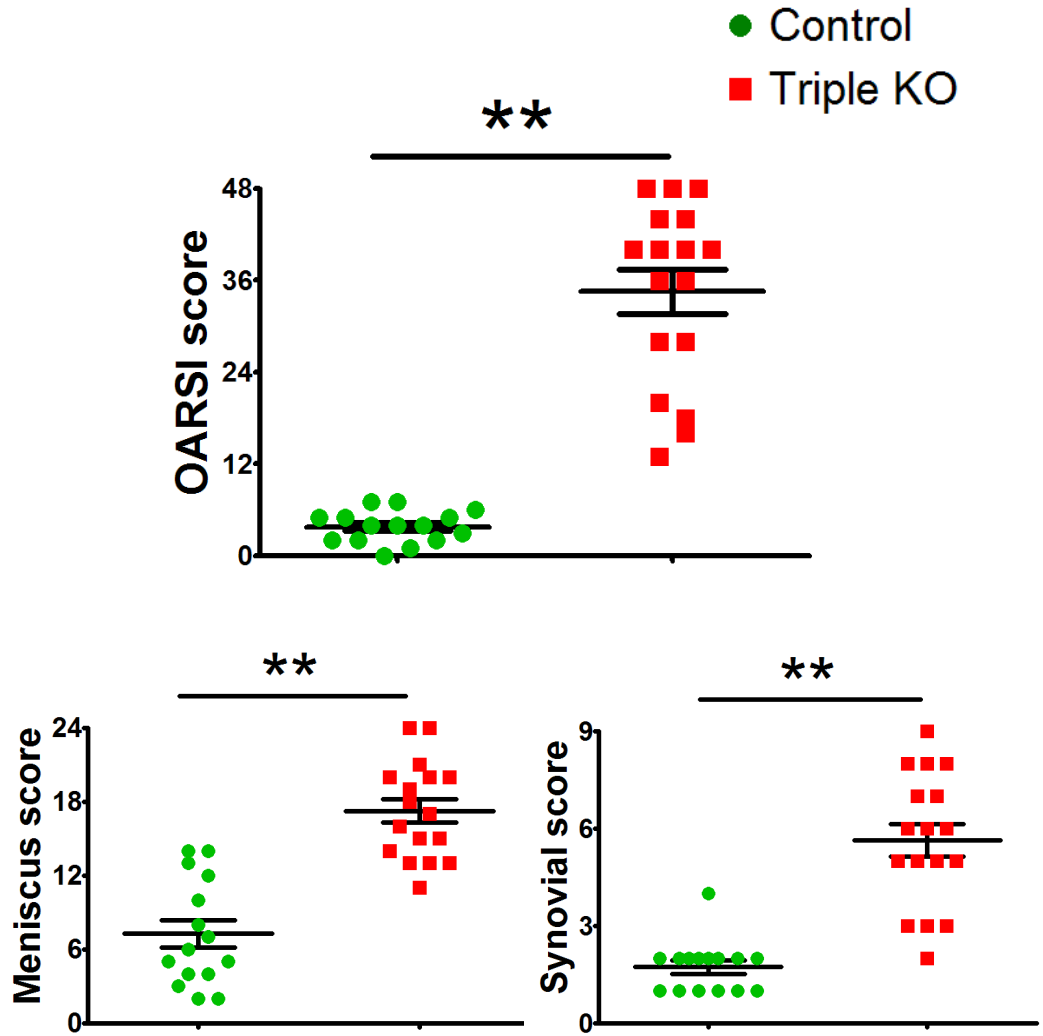
4 months of age

Treadmill: 6W
DMM surgery: 4,8W

Spontaneous OA model

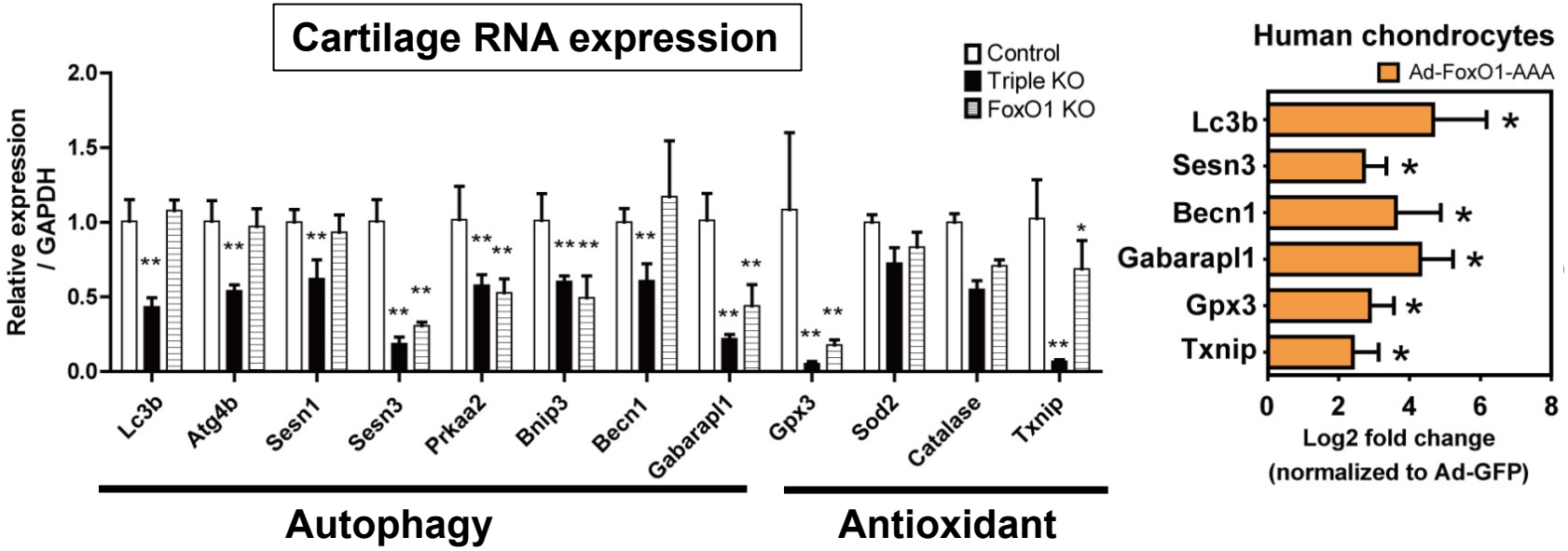
Spontaneous OA in Aggrecan-Cre-ERT FoxO TKO mice





Matsuzaki et al. Sci Transl Med. 2018

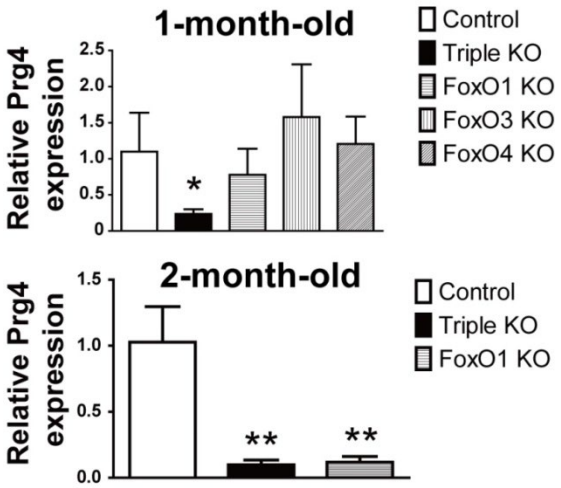
Mechanisms of cartilage damage in FoxO KO mice: Autophagy and oxidant defense



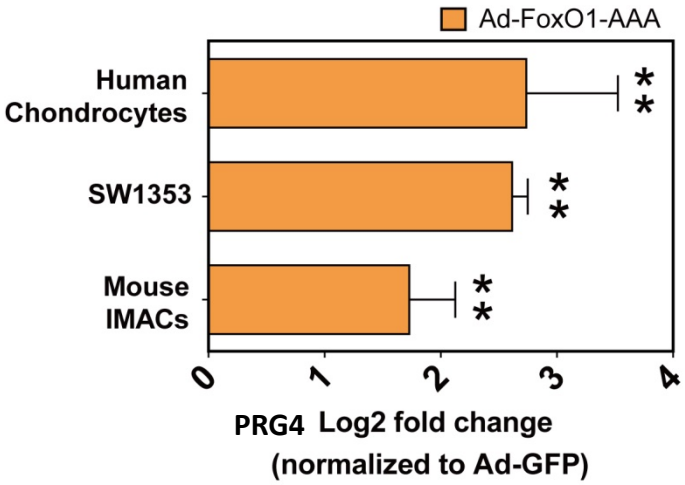
Matsuzaki et al. Sci Transl Med. 2018

Mechanisms of cartilage damage in FoxO KO mice: FoxO1 regulates *Prg4* expression

FoxO-deficient mice

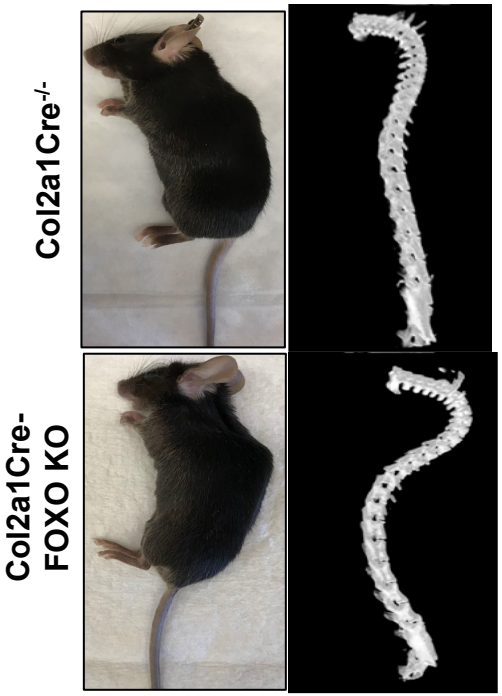


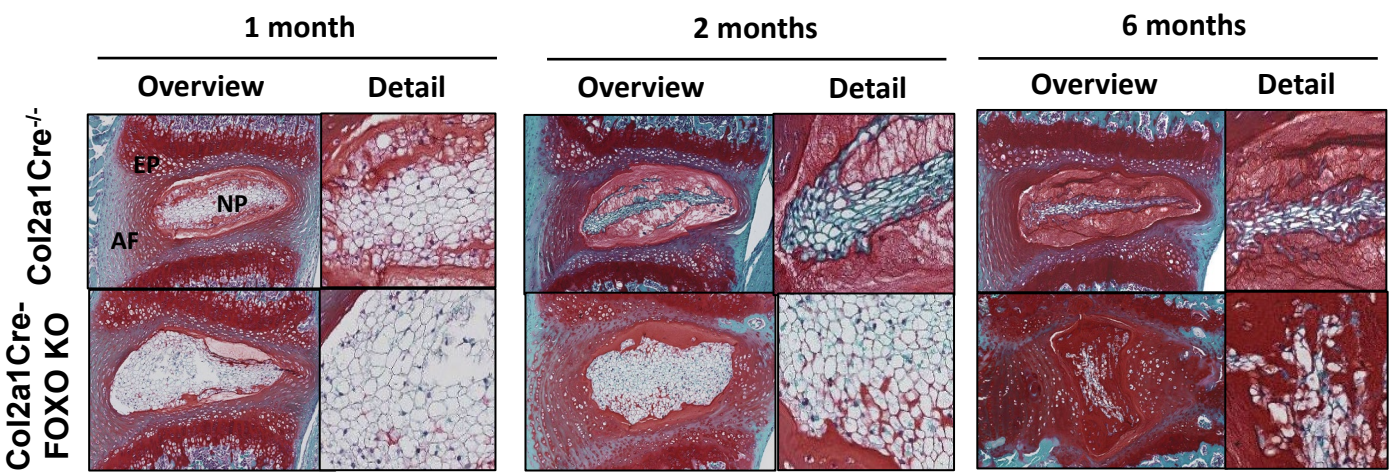
Overexpression of FoxO1

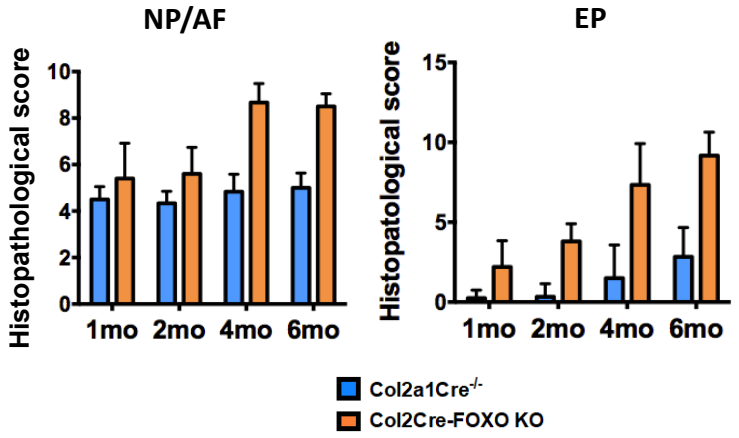


Matsuzaki et al. Sci Transl Med. 2018

Spontaneous intervertebral disc degeneration in mice with conditional deletion of FOXO







Alvarez-Garcia et al. Aging Cell 2018

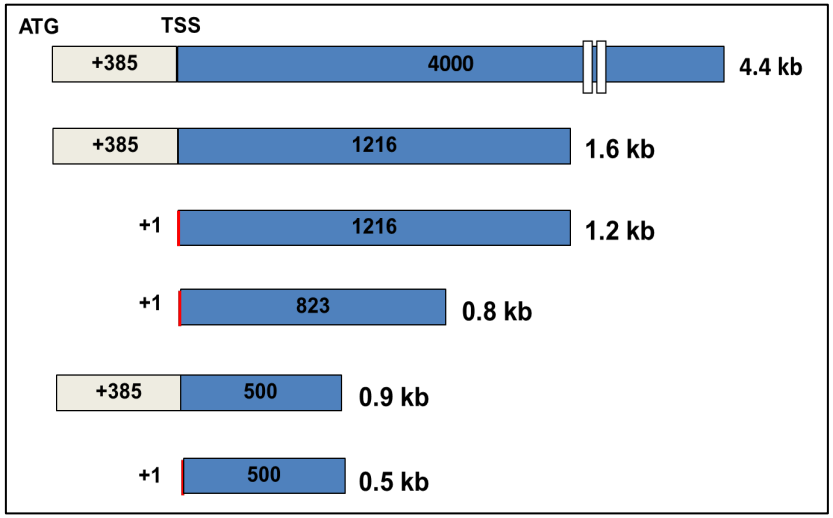
Summary

- FoxO1 and FoxO3 are suppressed in OA cartilage, meniscus and degenerated intervertebral disc.
- FoxO-deficient mice mice develop spontaneous cartilage degradation.
- Autophagy and antioxidant genes in cartilage cells are controlled by FoxOs.
- FoxO1 enhances PRG4 gene expression.
- Ad-FoxO1 normalizes gene expression in OA chondrocytes.
- FoxO1 and FoxO3 deficient mice spontaneously develop meniscus and intervertebral disc degeneration.

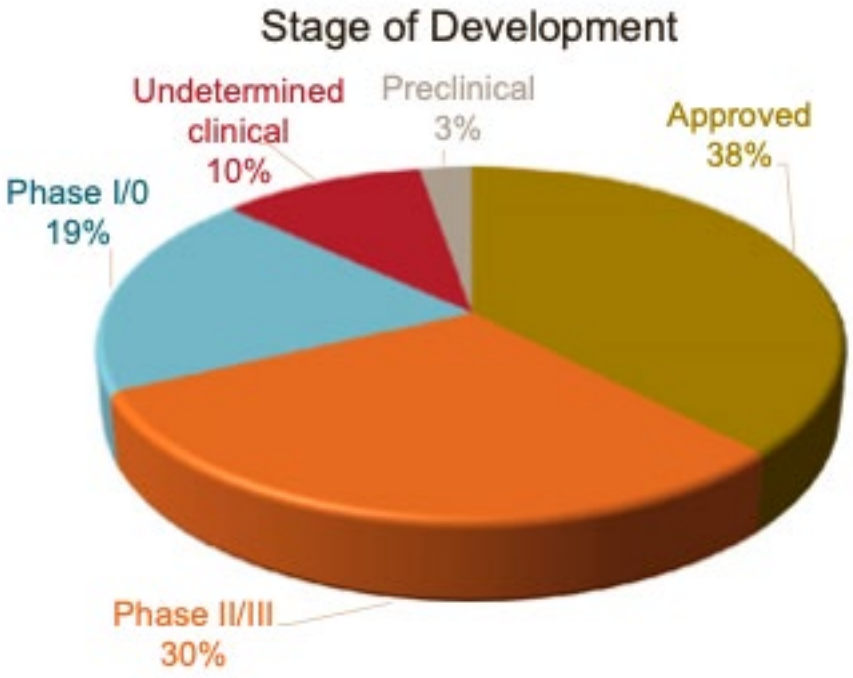
➢ FoxO are potential therapeutic targets for OA and intervertebral disc degeneration.

Drug screening for FoxO1

FoxO1 promoter constructs



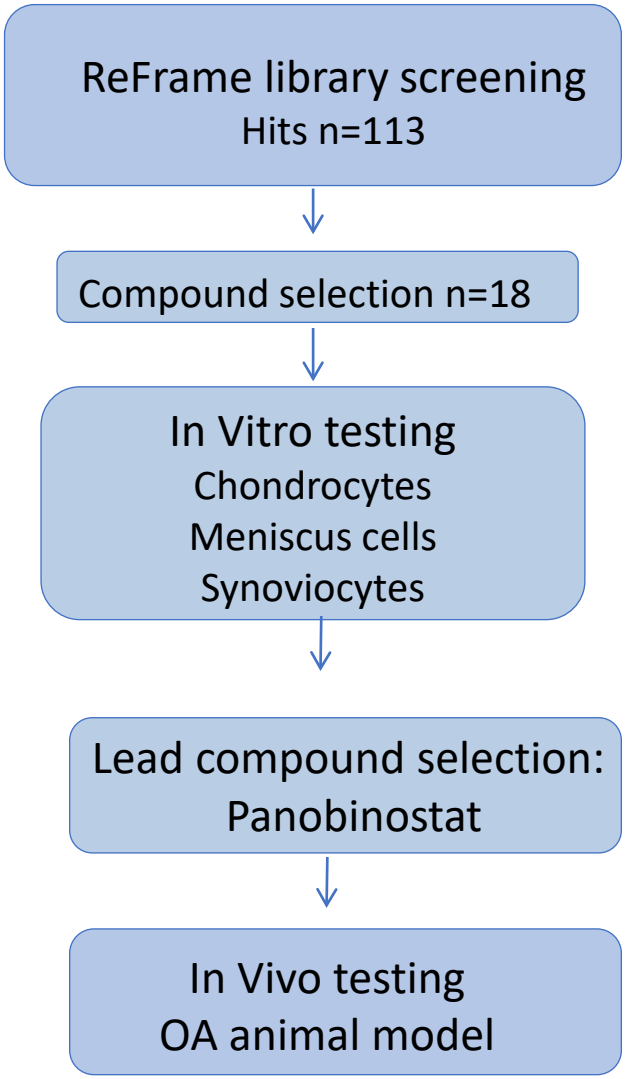
ReFRAME Library of ~12,000 compounds for drug repositioning



Created by CALIBR at Scripps Research

Janes at al. PNAS 2018

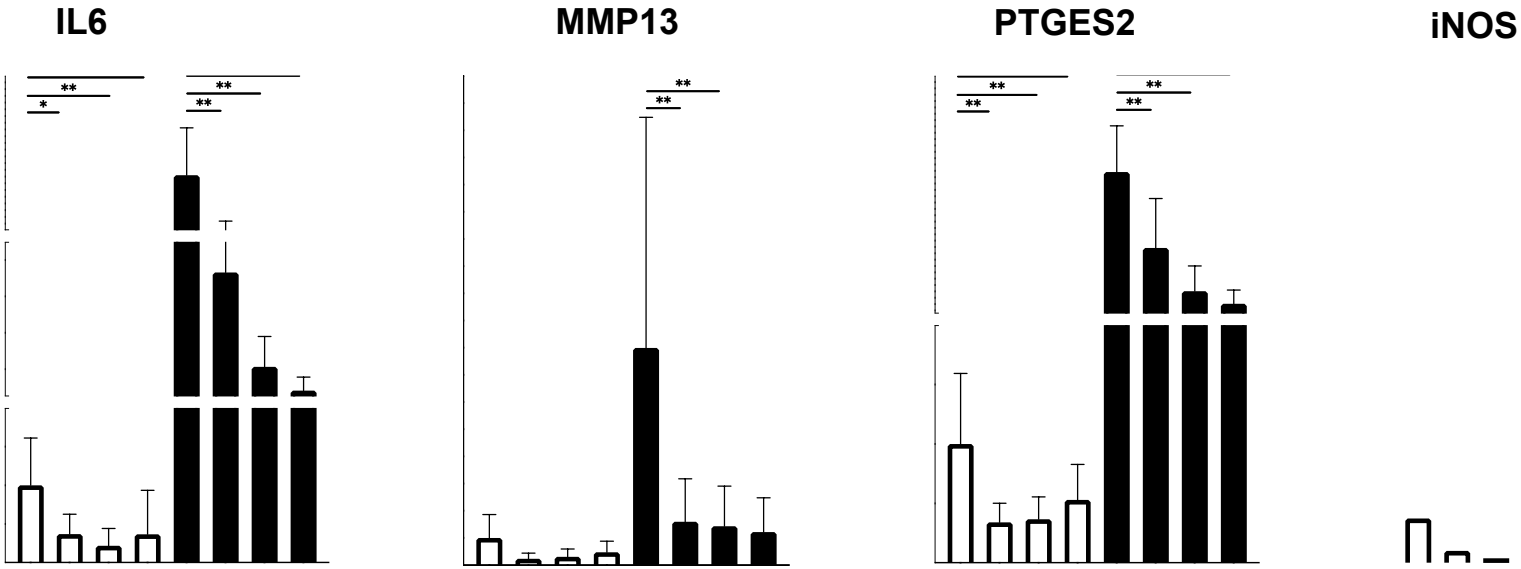
Drug screening for FoxO1

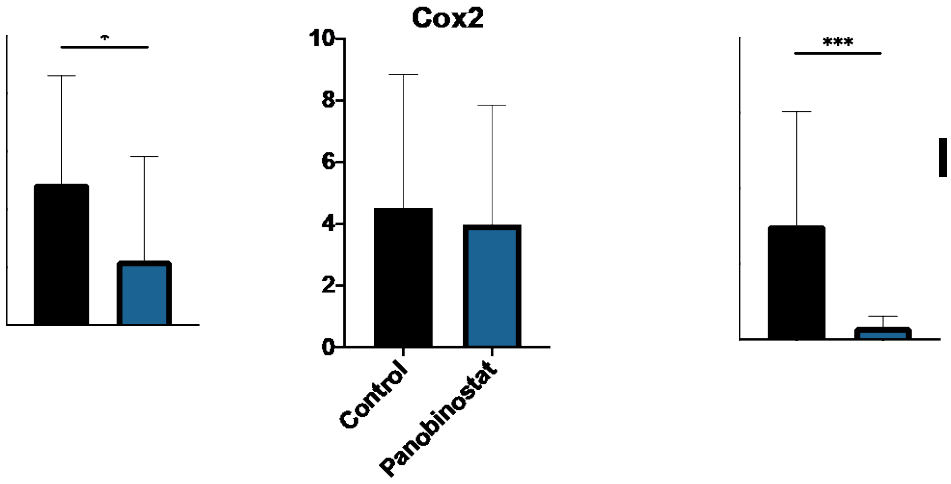


Panobinostat

- Histone deacetylase (HDAC) inhibitor
- HDACs have important functions in transcription regulation and in protein modification
- Pan HDAC inhibitor with potent activity against Class 2a HDACs
- More favorable in vitro profile than 3 other pan HDAC inhibitors

Panobinostat in human OA chondrocytes



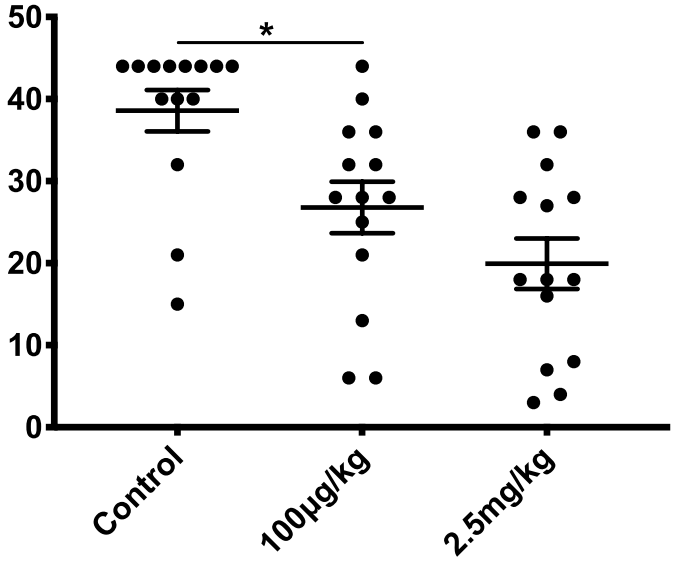


Injection in mouse knee joints

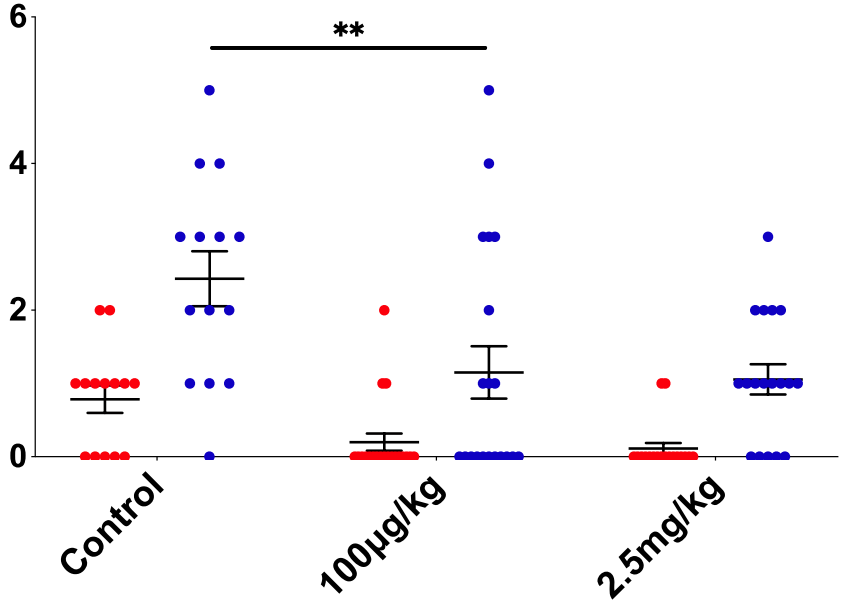
Ohzono et al. under review

Panobinostat reduced joint damage and pain in mouse OA model

Cartilage damage



Pain behavior



Red 1g filament
Blue 5g filament

Ohzono et al. under review

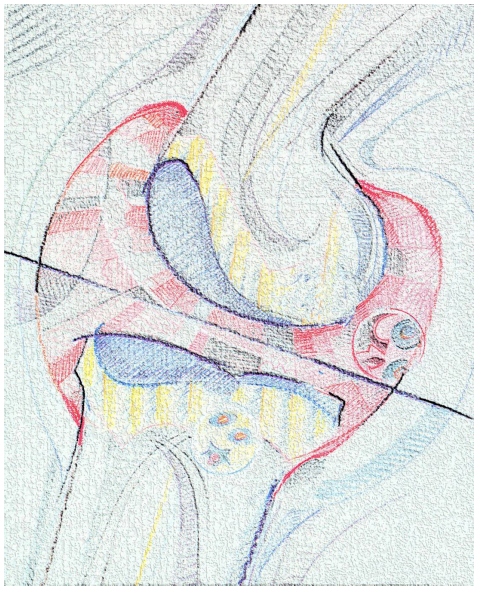
Summary

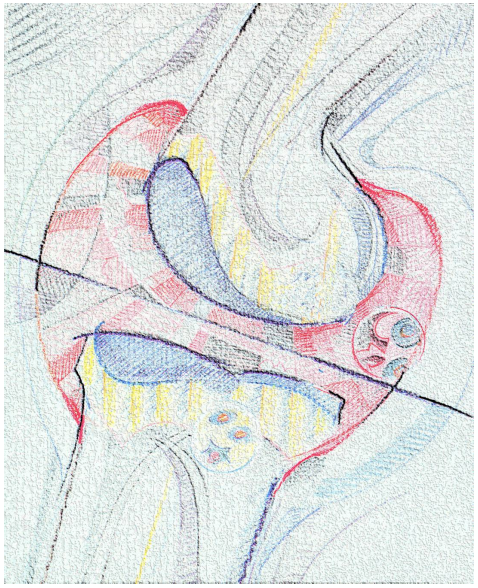
- HDAC inhibitors were among the top hits in high throughput drug screening of ReFRAME library with FoxO1 promoter reporter.

- Panobinostat was the most promising compound in testing of candidates in human chondrocytes, meniscus and synovial cells.

- In an animal model of experimental OA, Panobinostat reduced the severity of histological changes in cartilage, synovium and subchondral bone and improved pain behaviors.

➢ Panobinostat has a clinically relevant activity profile and is a candidate for OA symptom and structure modification.



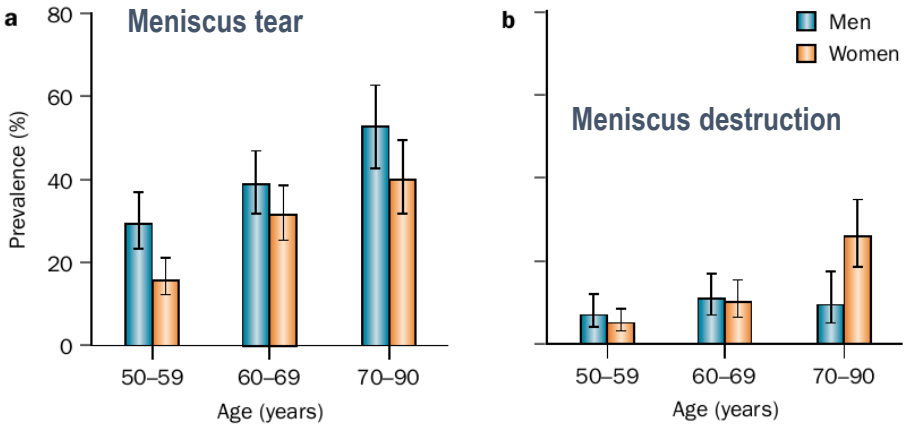


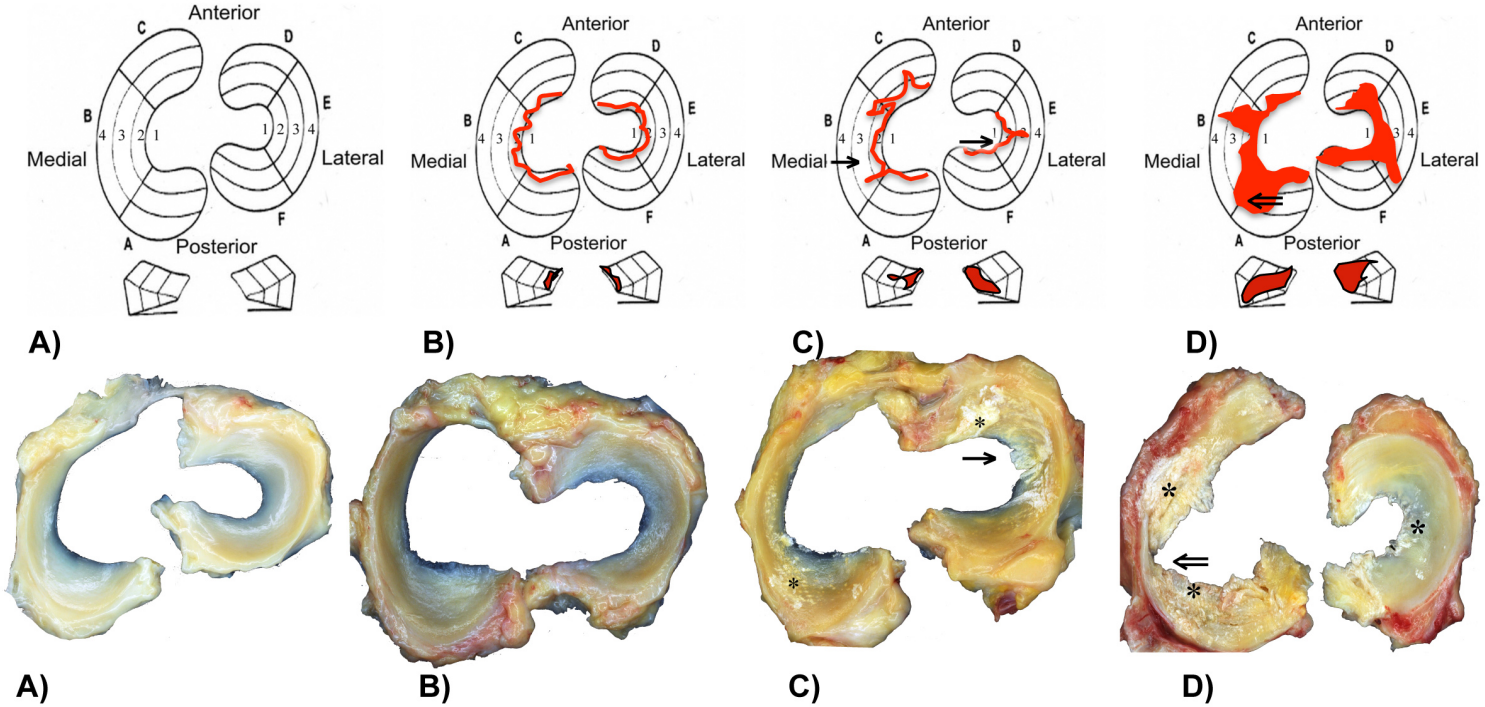
Osteoarthritis

- Disease impact and mechanisms of disease
- Drug target discovery
- FoxO transcription factors for Osteoarthritis therapy
- **Mohawk transcription factor for meniscus healing and Osteoarthritis prevention**

Meniscus destruction and OA

- Menisci are essential to the biomechanical function of the knee joint
- Acute meniscus injury can lead to Post-traumatic osteoarthritis (PTOA)
- Aging-associated degenerative meniscus tears are highly prevalent and promote OA progression





Targeting meniscus destruction and OA

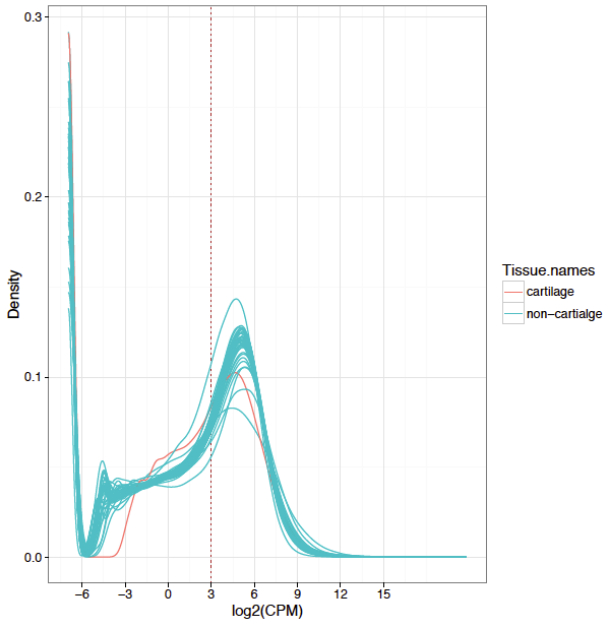
Discover key regulators of meniscus cell identity and normal function by comparing transcription factors that are enriched in meniscus compared to other human tissues

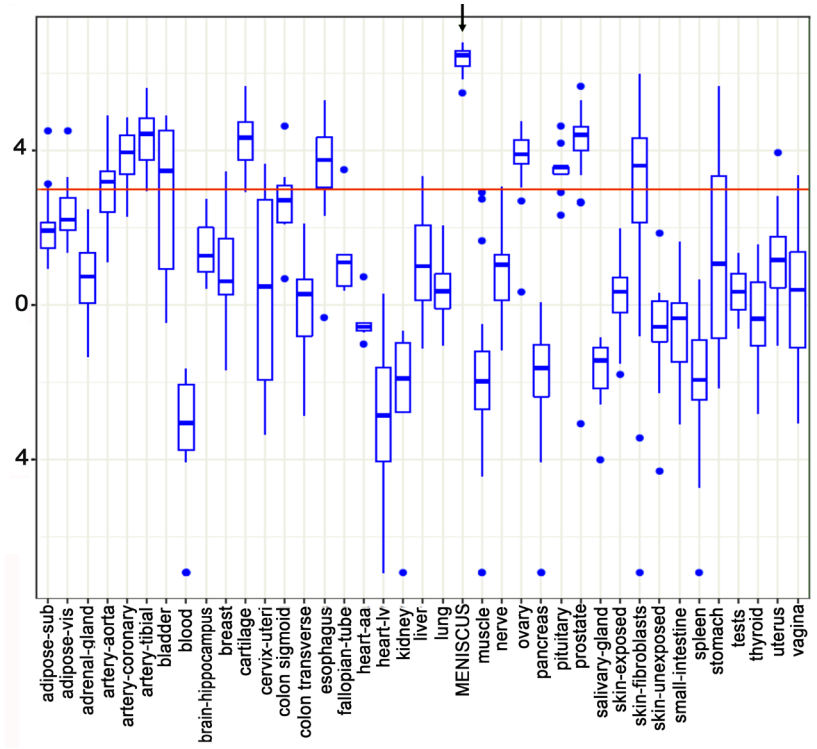
Test candidate in cell and tissue models of meniscus injury

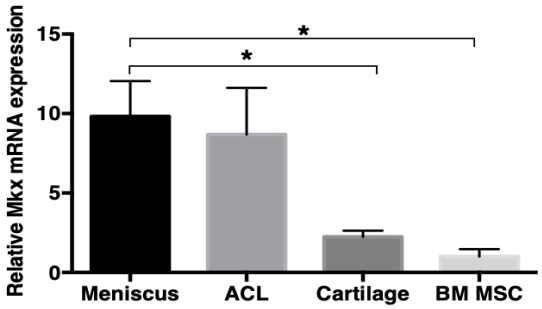
Test candidate in animal model of meniscus injury and osteoarthritis

Mohawk (MKX) is the most meniscus-enriched transcription factor

GTEx Portal







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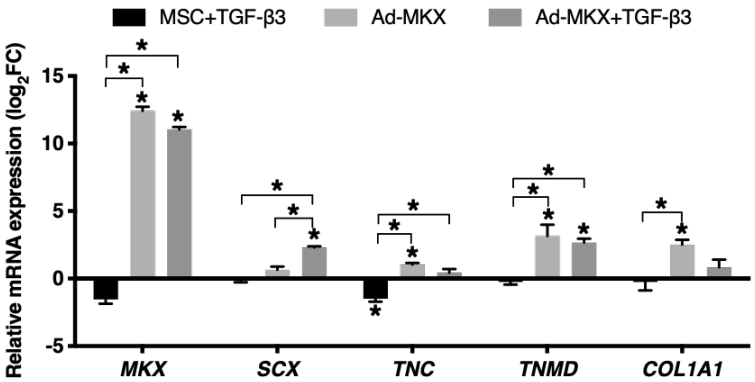
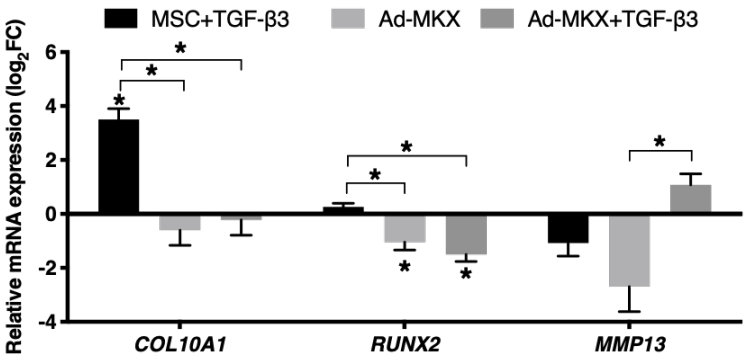
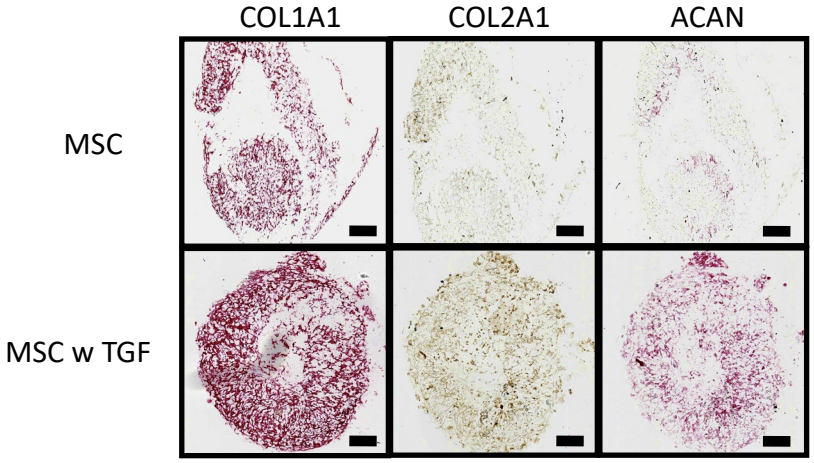
Mohawk plus TGF-β3 induces stem cell differentiation to a meniscus cell

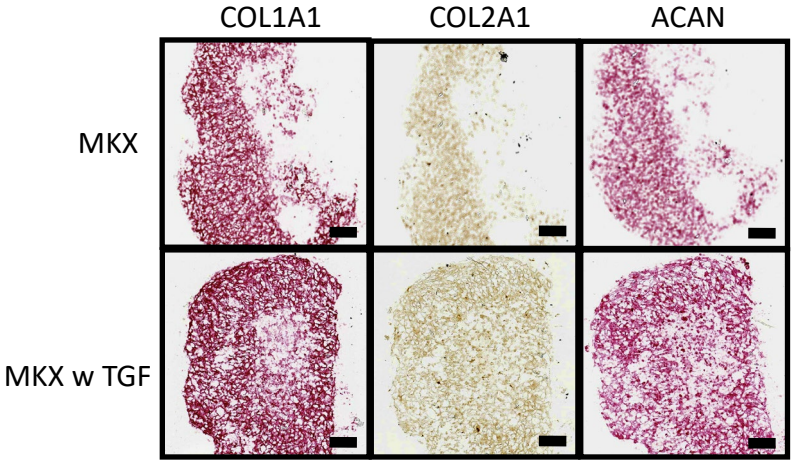


Mkx and fibrogenic differentiation

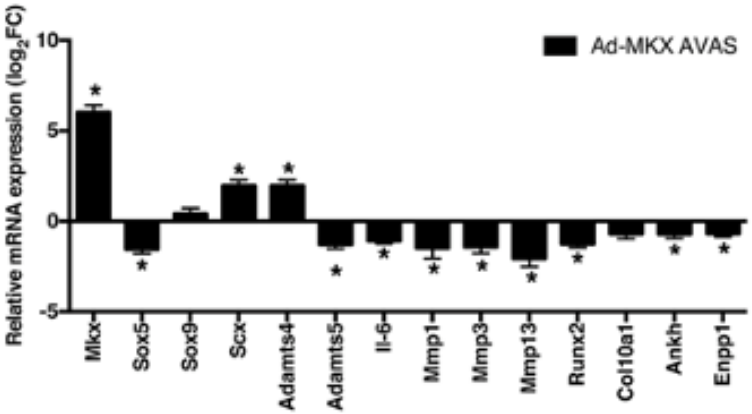


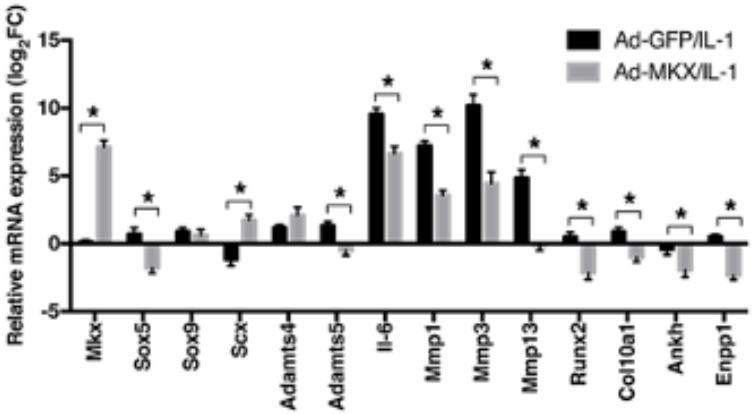
Chondrogenic differentiation





Mohawk suppresses genes that promote meniscus damage





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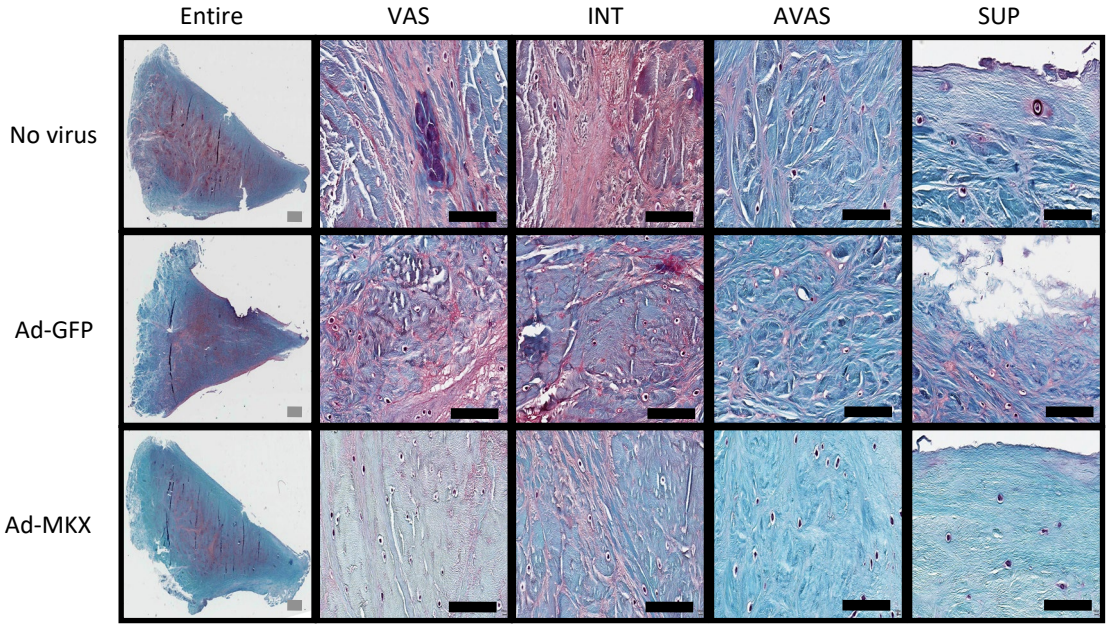
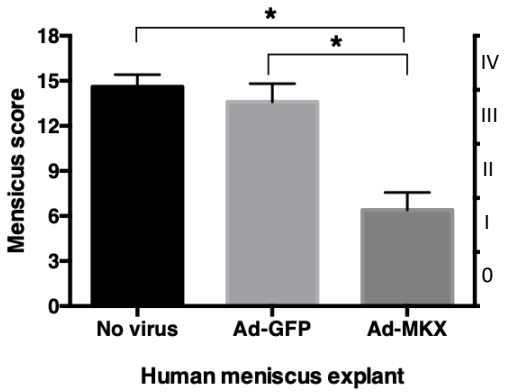
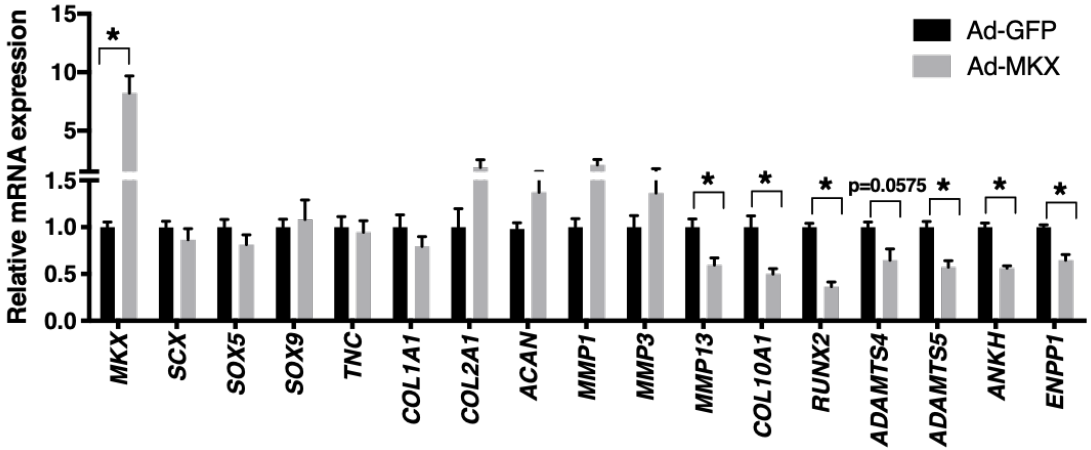
Ad-MKX promotes meniscus repair and suppresses genes associated with meniscus calcification and ECM degradation in human explants







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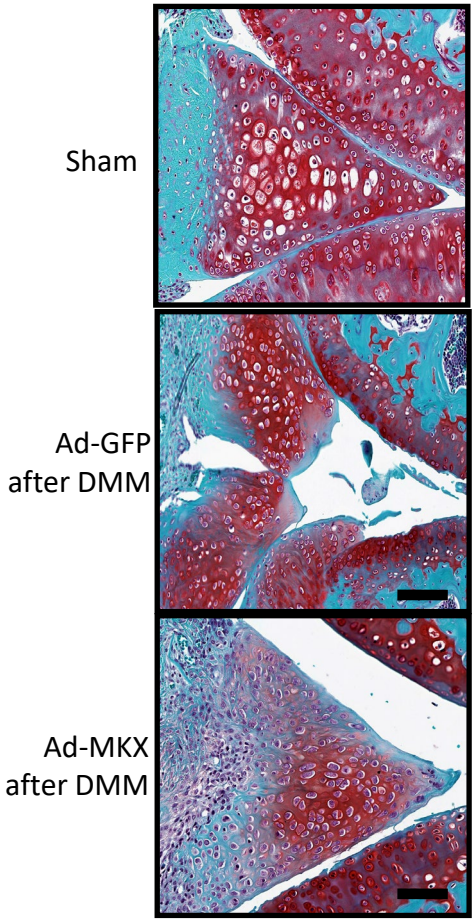
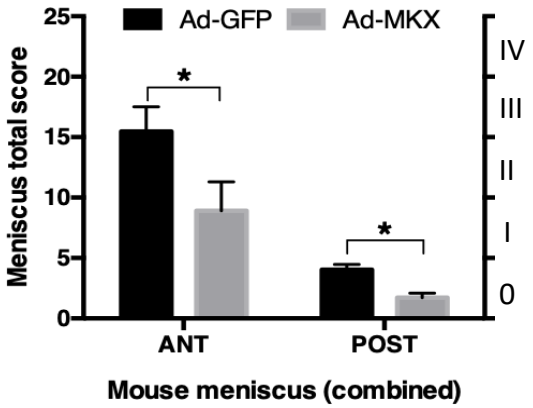
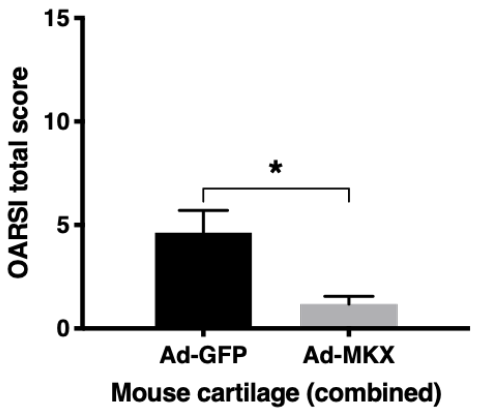
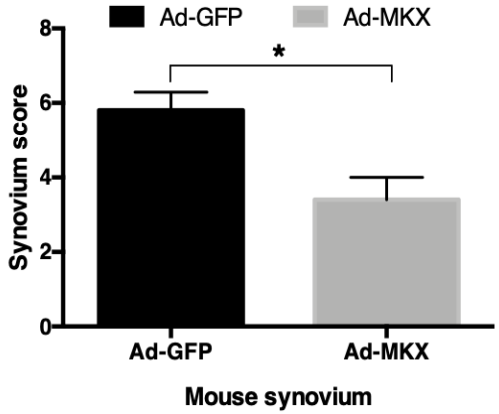
Ad-MKX injection into mouse knees protects against meniscus and cartilage damage induced by surgical meniscus destabilization









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Summary

- MKX is the transcription factor that is most enriched in meniscus.
- MKX induces differentiation of mesenchymal stem cells to a meniscus cell phenotype.
- In menisci from human OA joints, Ad-MKX promotes meniscus repair and suppresses genes associated with meniscus calcification and ECM degradation.
- In a mouse model of meniscus injury, intraarticular Ad-MKX injection leads to healing of experimental meniscus tear and reduces cartilage damage.

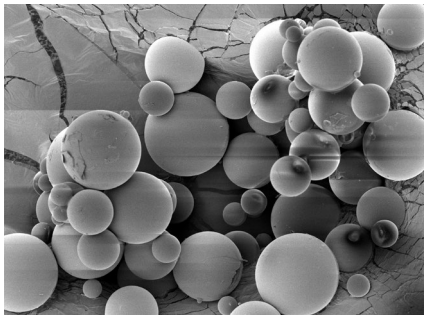
➢ MKX gene delivery is a promising approach to prevent post-traumatic OA and slow progression of aging-related OA.

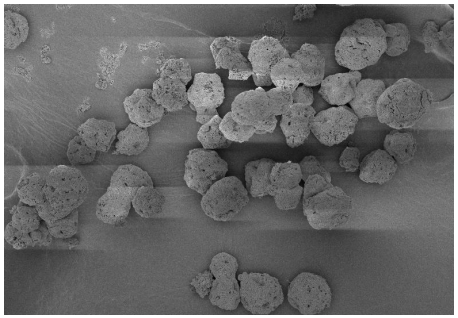
Ongoing studies

Develop local therapies for injections into joints and intervertebral discs

Sustained release formulations for small molecules: PLA/PLGA Microspheres

Day 1

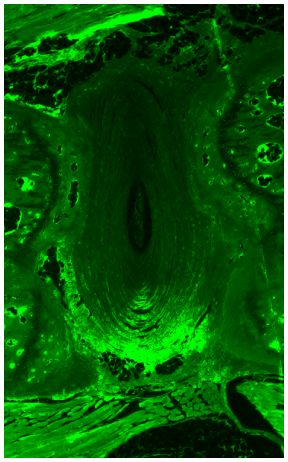


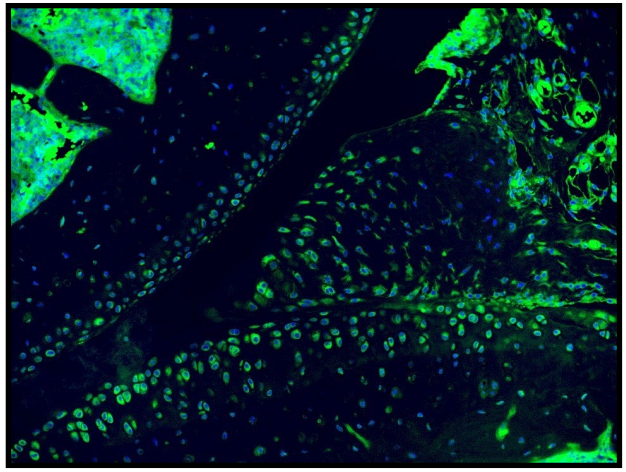


Week 26

AAV-mediated gene delivery

AAV-GFP
Rat
Intervertebral disc





AAV-GFP
Mouse knee

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Philip Ordoukhanian

Tomas Duffy

Hannah Swahn

Spine

Oscar Alvarez-Garcia

Jasmin Mertens

Kevin Myers

Hiroshi Asahara

Darryl D'Lima

Merissa Olmer

Connie Choate

KLF transcription factors

Manabu Kawata

Gene variant for pain

Alice Courties

Meniscus

Jason Lee

Drug discovery

Kristen Johnson, Calibr

Panobinostat

Hiroki Ohzono

Yiwen Hu

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UPCOMING LECTURE

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Julia Moore Vogel, MBA, PhD
Program Director, The Participant Center,
All of Us Research Program
Scripps Research Digital Trials Center

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Wednesday, December 15

1:00 PM PT/4:00 PM ET

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