# Understanding FOP – an Exciting Journey

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# FOP – a rare disease

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# The value of studying rare disease

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- Important for therapeutic development for those directed affected
- Provide unique insights: reveal genes, signaling pathways, mechanisms with key biological importance

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What is learned through rare disorders can be applied to more common related conditions that are often more difficult to study

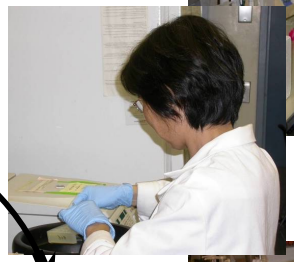
- provide opportunities to develop treatments and prevention







bedside
bench


# Fibrodysplasia Ossificans Progressiva (FOP)

A rare human genetic disorder

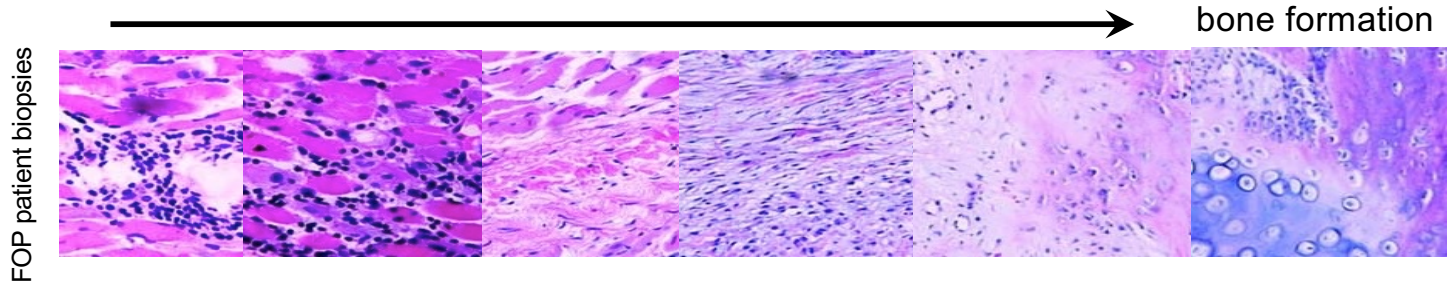
- Population frequency* = ~1 per 1 million people

Inheritance

- Most cases are sporadic (new/single case in a family)
- Autosomal dominant (only a few known cases of genetic transmission)

Clinical presentation

- Some malformations of the skeleton
- Extensive, progressive heterotopic (extra-skeletal) bone




*Current estimated prevalence: 0.6-1.39 in 1 million people; ~900 confirmed cases worldwide
Baujat et al., OJRD 2017; Pignolo et al., OJRD 2021; IFOPA website https://www.ifopa.org/

# The search for mutations that cause FOP

Prior to genome-wide sequencing technologies, **genetic linkage** studies and **positional cloning** were standard strategies to identify the responsible genes for genetic diseases.

- These approaches depend on acquiring genomic DNA samples from families, preferably multi-generational families, with inheritance of the disease.
    - **Clinical criteria** to identify people with FOP needed to be clearly defined.

- Genetic linkage looks for co-inheritance of specific genome regions with the disease, and positional cloning identifies the causative gene within the linked region.

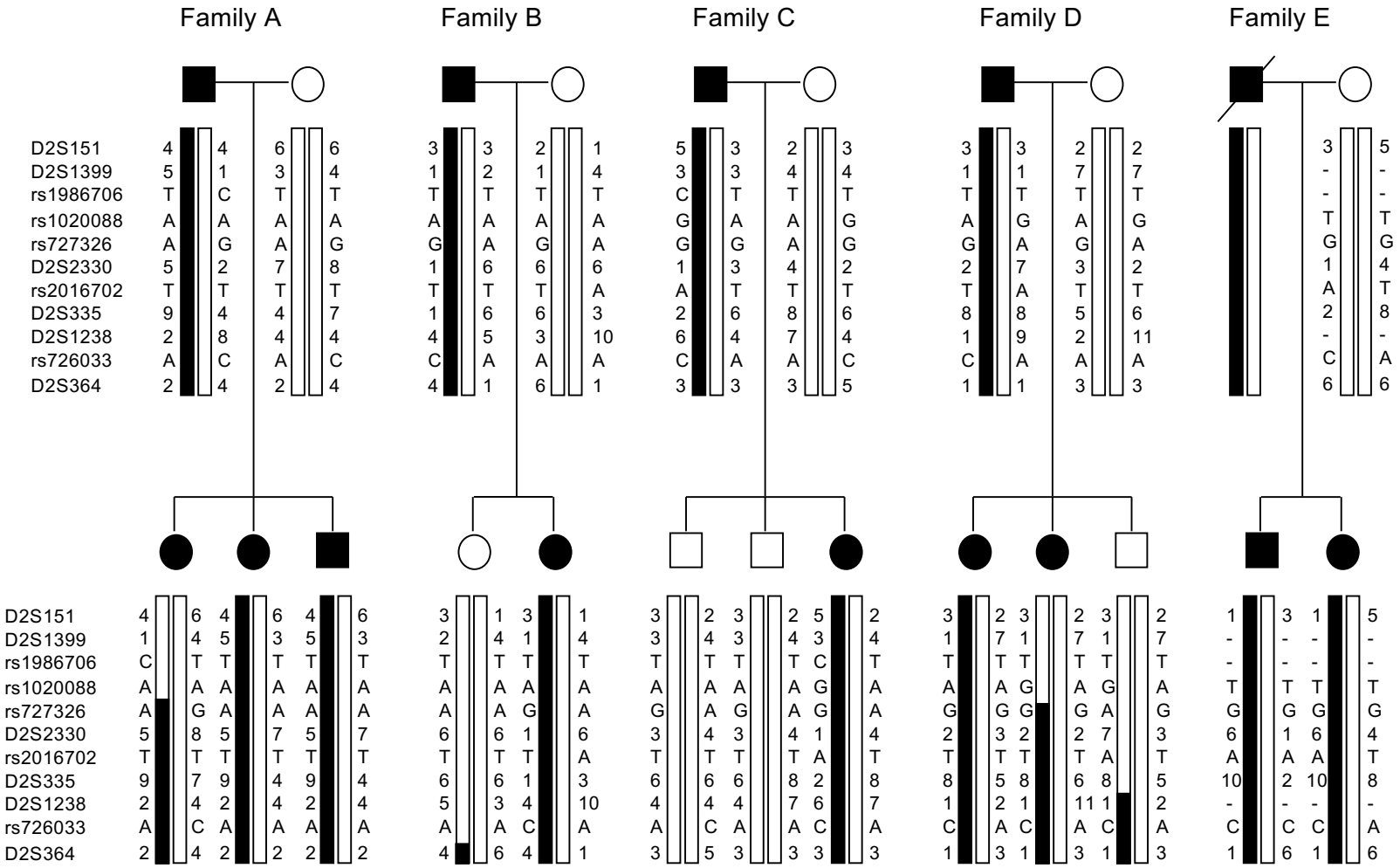
Challenges of a rare disease like FOP:

Most cases are sporadic (*de novo* mutations).

Only a few cases of genetic transmission within families could be identified.

- FOP linkage analysis was conducted with five two-generation families.

# Linkage Analysis: FOP is linked to a region of chromosome 2



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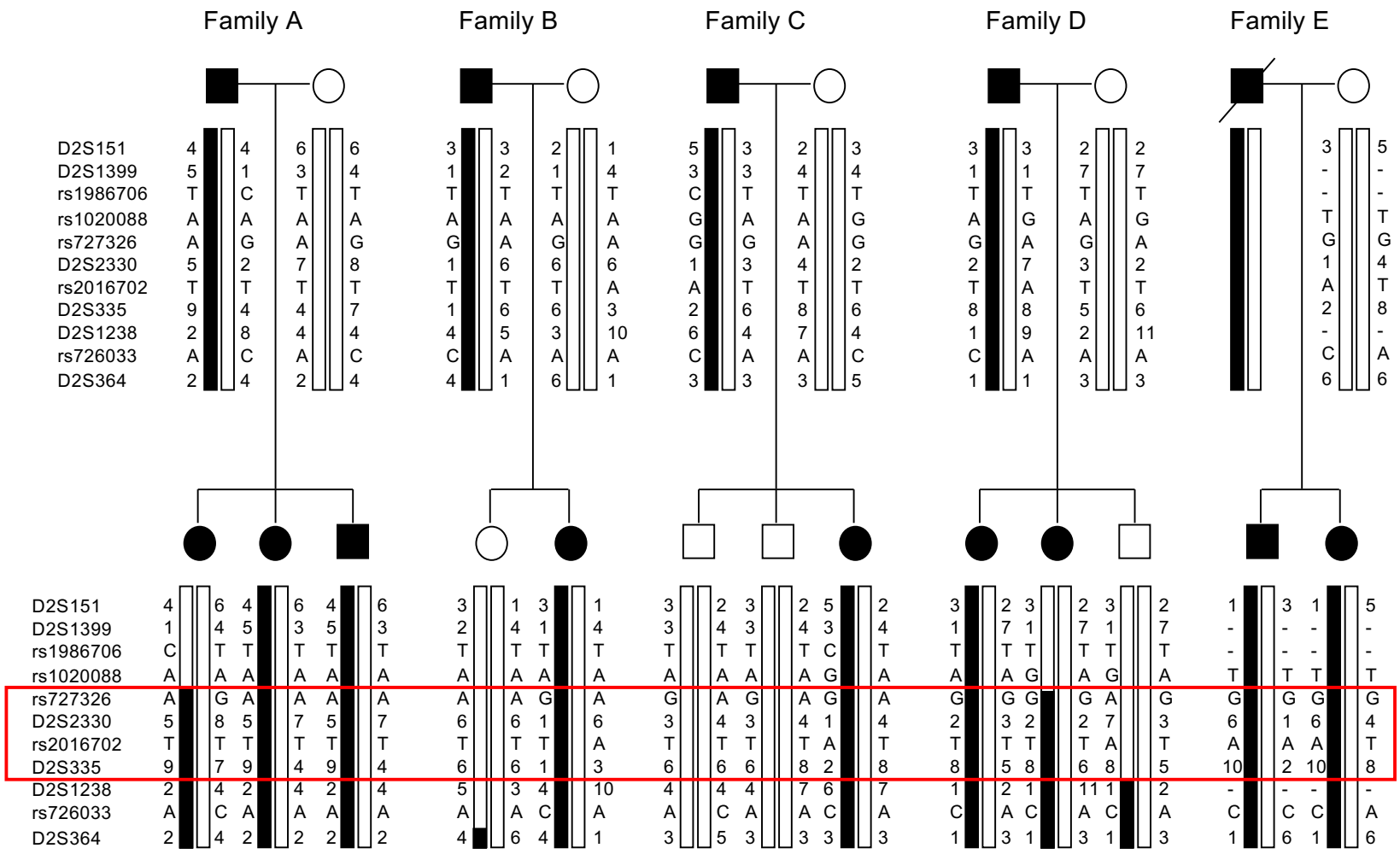
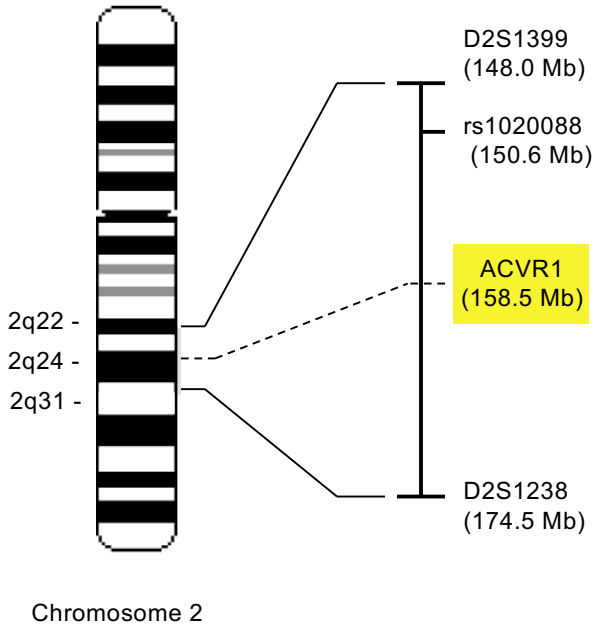


# Genetic Linkage Analysis in FOP

- Genome-wide linkage analysis used five families clinically diagnosed with ‘classic’ FOP.
  - Linkage to chromosome 2q23-24 was identified.
    - No other genomic region showed consistent linkage in all five families.

    - A primary candidate gene within the linked region was *ACVR1*, a type I receptor in the BMP signaling pathway




Shore et al., Nature Genetics 2006

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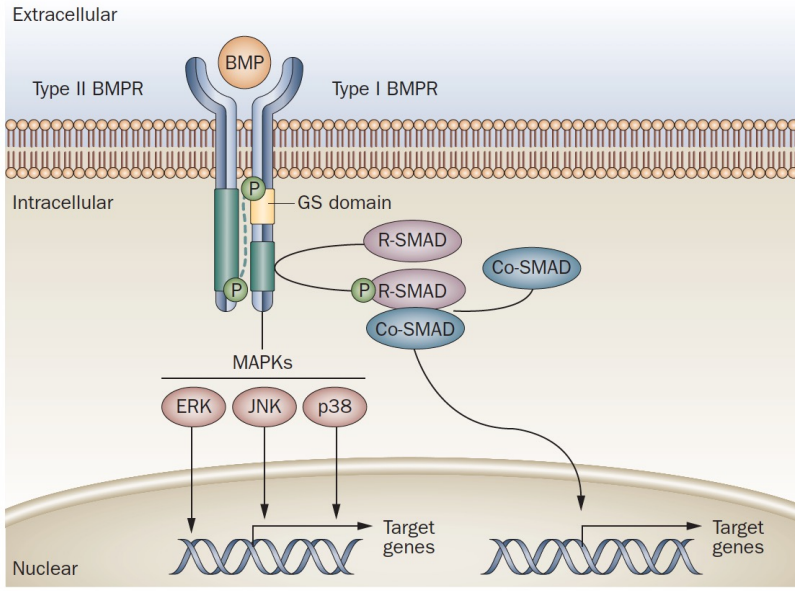
    - Our studies had shown that BMP pathway signaling is altered in FOP cells.

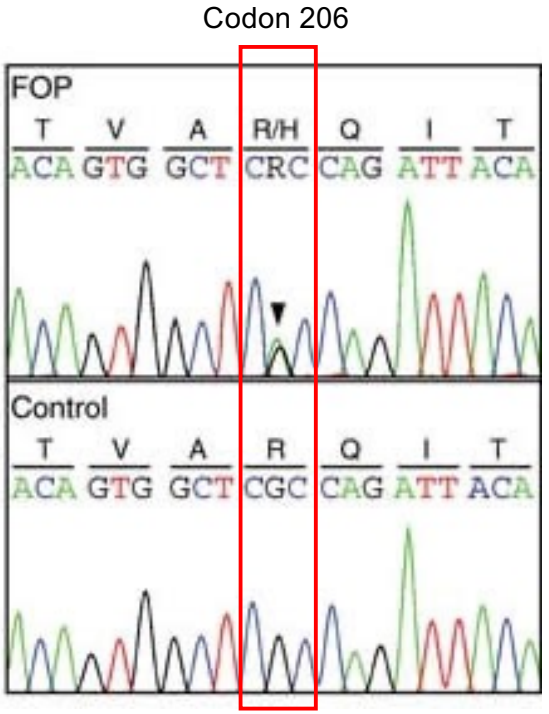



Shore and Kaplan, Nat Rev Rheum 2010

# Mutation screening for *ACVR1* - Classic FOP

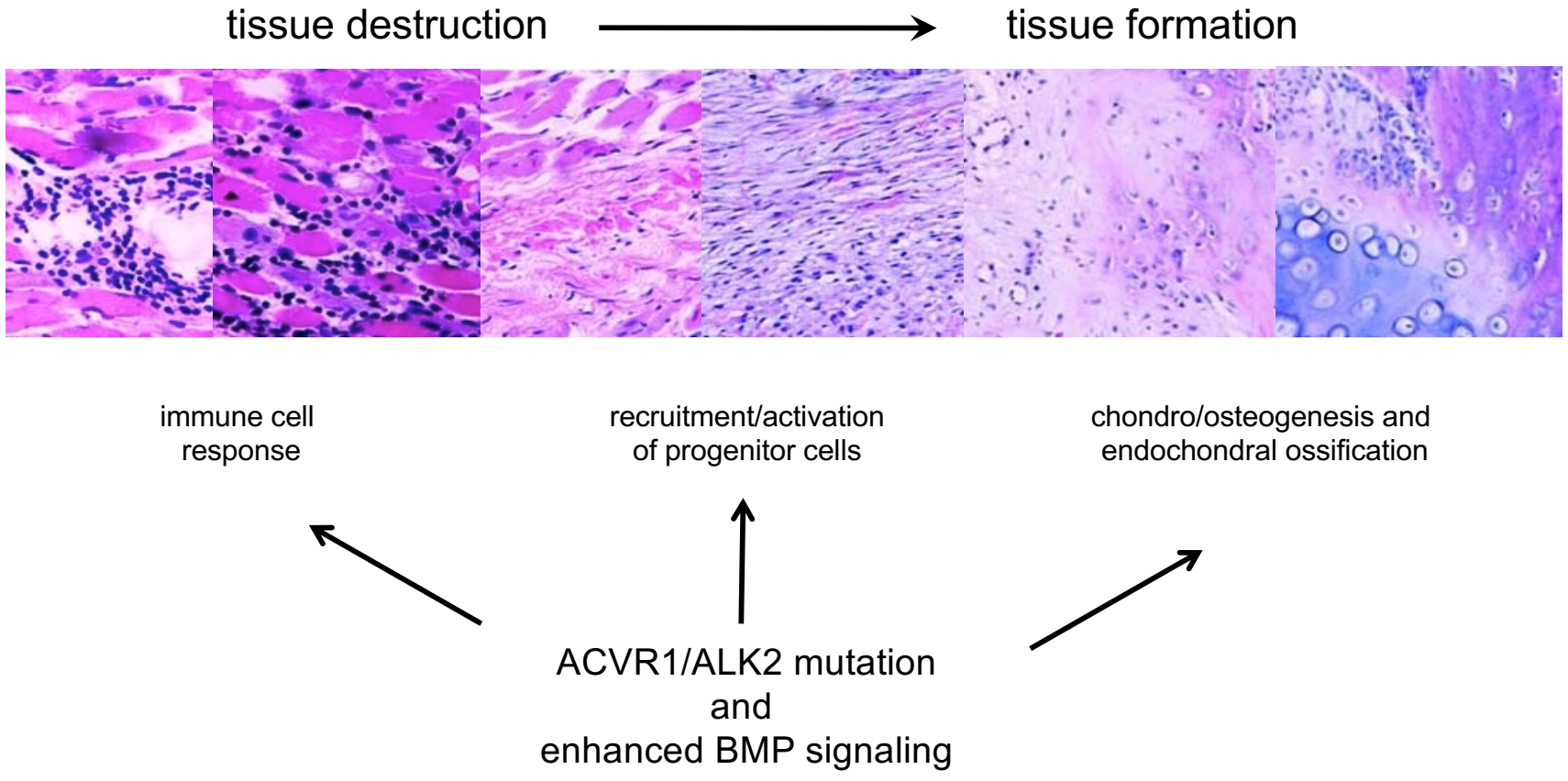
- The identical heterozygous single nucleotide substitution in the *ACVR1* gene was identified in all patients (sporadic and familial) with classic FOP.
  = c.617G>A; R206H

- The c.617G>A nucleotide mutation is not found in any unaffected individuals
  - this mutation is fully penetrant (no unaffected carriers)



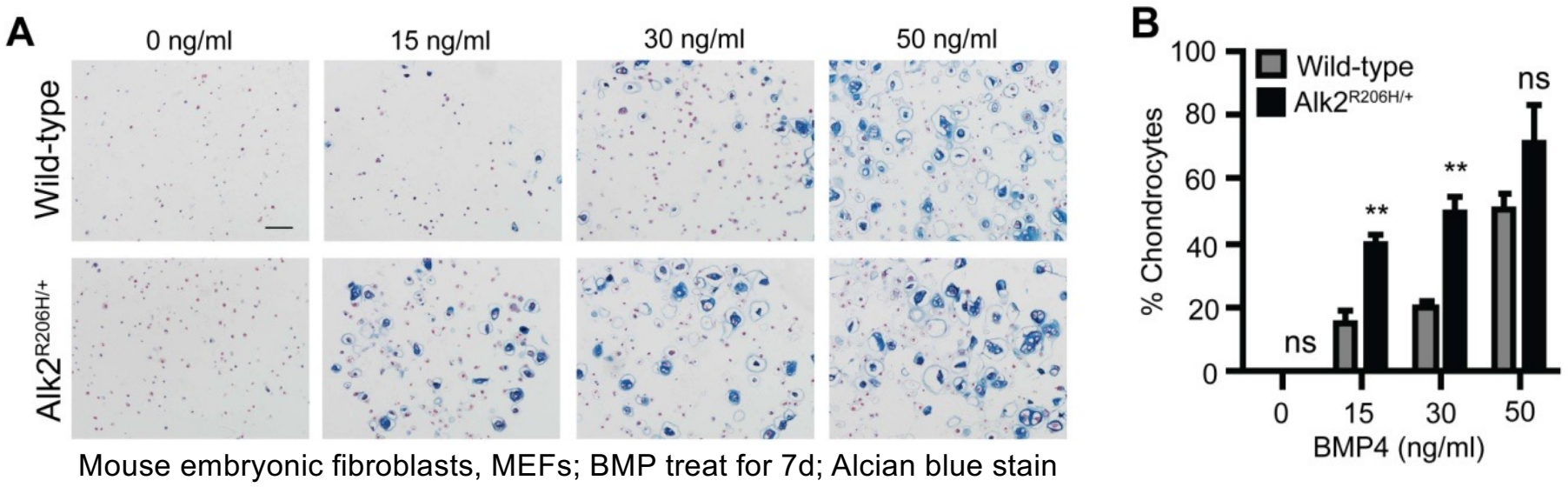

Shore et al., Nature Genetics 2006

# Examine the impact of the ACVR1 mutation at multiple steps during formation of heterotopic ossification (HO)



# *Acvr1*$^{R206H/+}$ mesenchymal cells more readily undergo chondrogenesis

*Acvr1*$^{R206H/+}$ mesenchymal cells show increased sensitivity to BMP-induced chondrogenesis and accelerated chondrogenic differentiation *in vitro*.




Mouse embryonic fibroblasts, MEFs; BMP treat for 7d; Alcian blue stain

Culbert et al, 2014 *Stem Cells*

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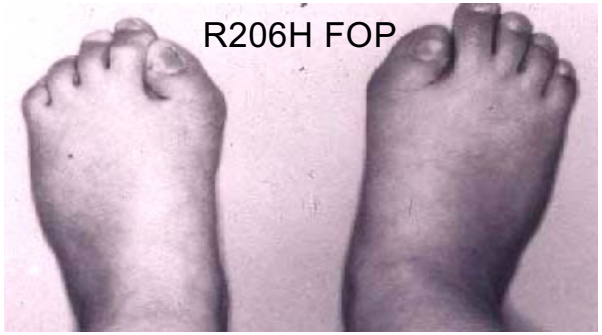
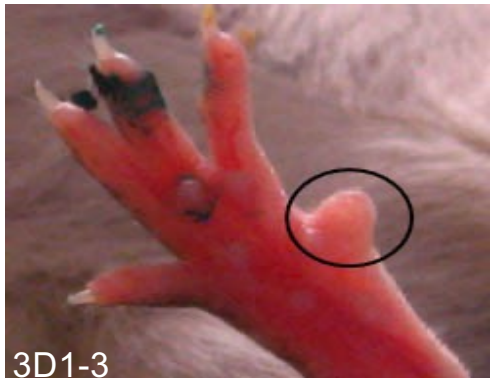
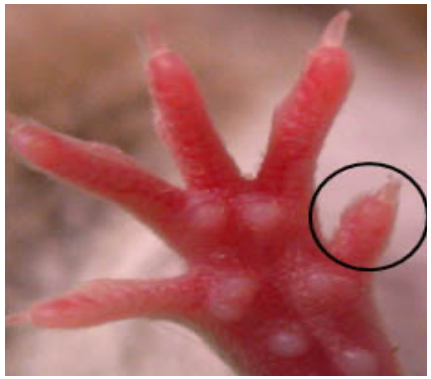
# *Acvr1*$^{R206H/+}$ knock-in mouse model for FOP

Toe malformation



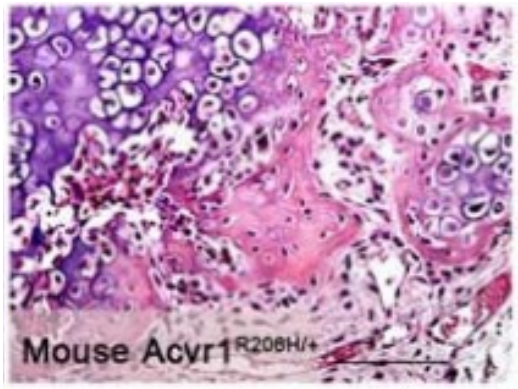




R206H



Control

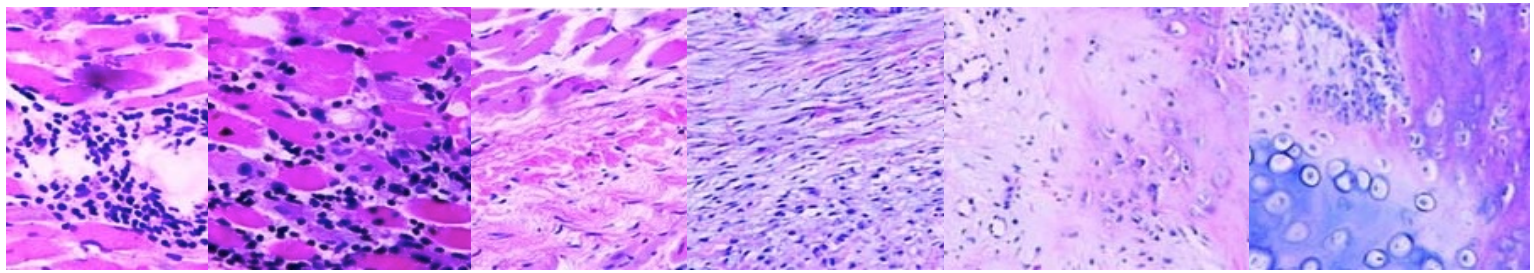
Heterotopic ossification (endochondral)

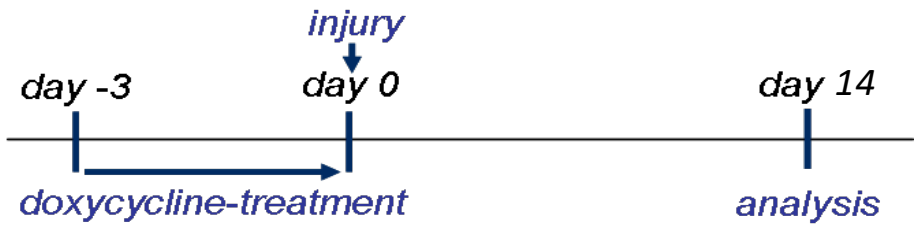

Chakkalakal et al., 2012 *JBMR*

# *Acvr1*$^{R206H/+}$ conditional knock-in mouse model of injury-induced HO

FOP patient biopsies



| Post-injury (skeletal muscle, cardiotoxin) | immune cell response | recruitment/activation of progenitor cells | chondro/osteogenesis and endochondral ossification |
|---|---|---|---|
| | days 1-3 | days 4-6 | days 7-14 |




*Acvr1*$^{R206H/+}$*;rt-tetO-Cre* mice

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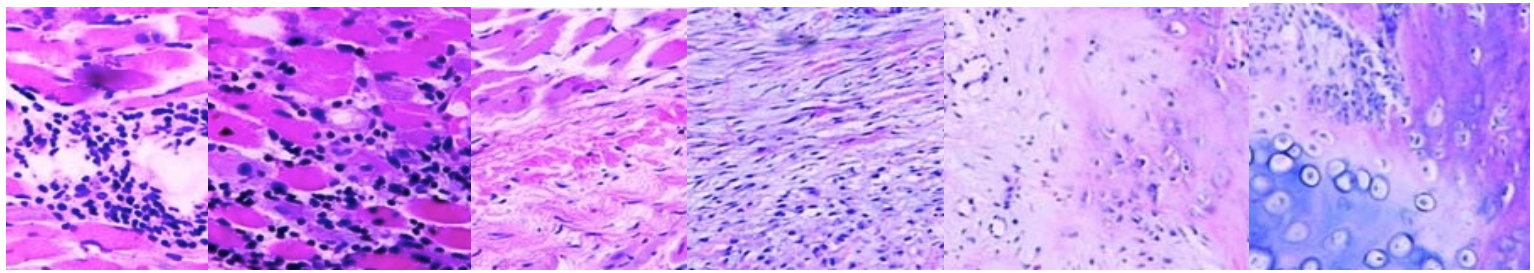

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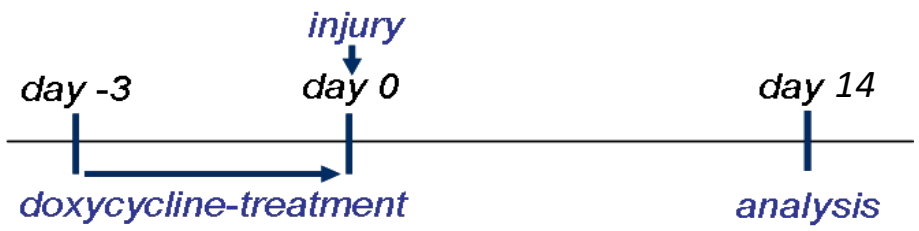


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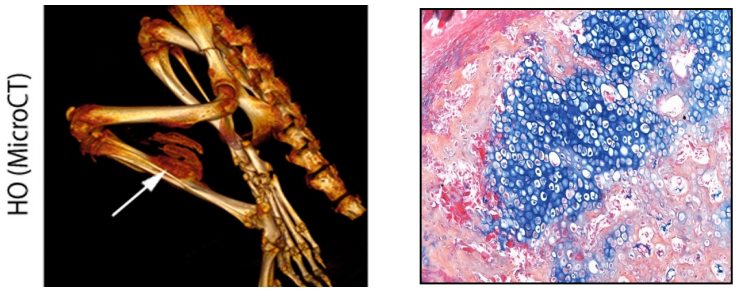
Day 14




200X

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The first steps in understanding FOP:

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- HO progenitor cell identities and fates
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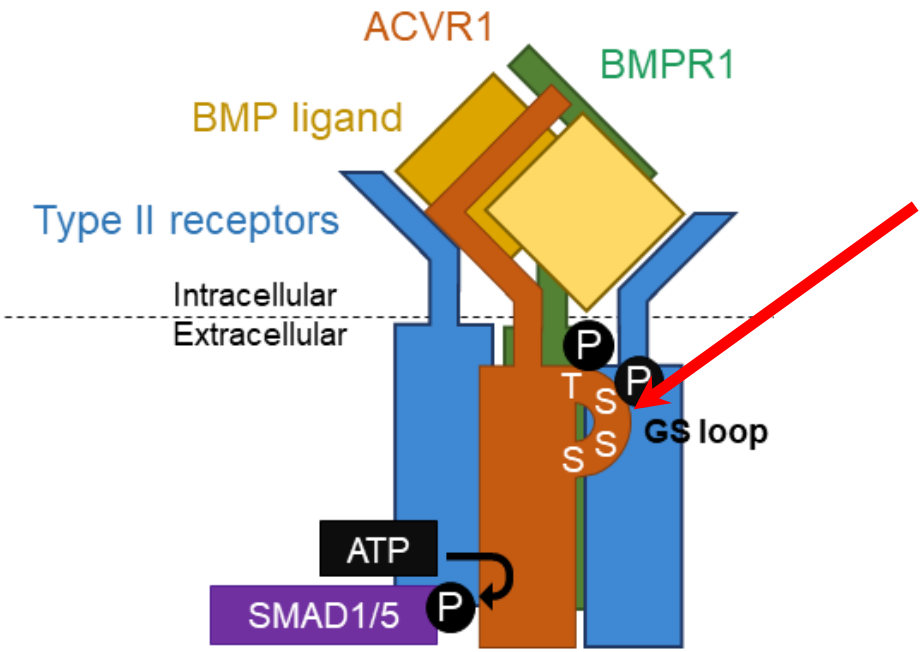
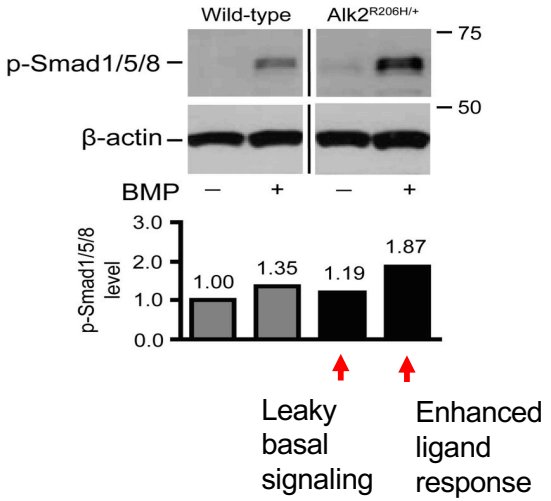
# Genetic mutation: Activating mutations in ACVR1/ALK2




ACVR1/ALK2 is a cell surface type I receptor in the TGFβ/BMP signaling pathway.

The common *ACVR1* c.617G>A mutation in FOP changes a single amino acid (R206H) in the GS domain, a key regulatory domain for downstream signaling.

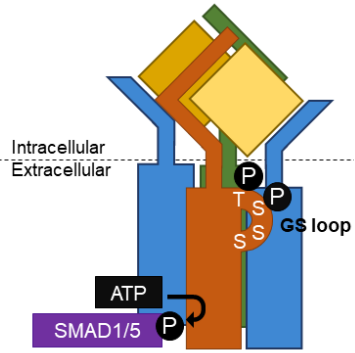
ACVR1 FOP mutations induce increased BMP pathway signaling.




Culbert et al, 2014 *Stem Cells*

# Genetic mutation: Molecular mechanisms

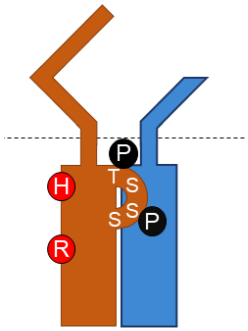
FOP-ACVR1 is no longer constrained by the usual molecular mechanisms that regulate activity of the BMP receptor complex.




Wild-type ACVR1

## Ligand

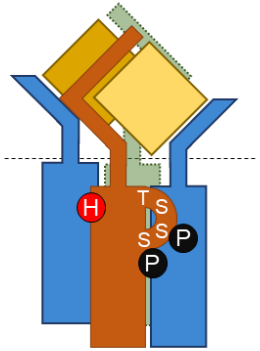
- Ligand-independent and ligand-responsive activity
  - Signaling in the absence of ligand
- Specificity
  - Activin A: acquired/increased responsiveness




ligand-independent
ACVR1-R206H
or -G328R

## Type I BMP receptors

- Reduced requirement for partner type I BMP receptors for signaling
- Reduced GS domain phosphorylation requirements
  - Influenced by ligand availability
  - Influenced by specific ACVR1 mutation (R206H, G328R)




ligand responsive
ACVR1-R206H

Culbert et al, 2014 *Stem Cells*; Hino et al, 2015 *PNAS*; Hatsell et al 2015 *STM*; Allen et al, 2020 *eLife;* Ramachandran et al, 2021 *EMBO J*; Yamamoto et al, 2022 *JBMR*; Allen et al, 2023 *JBMR*

# Our bodies are composed of multiple tissues and organ systems that use specific types of cells for specialized functions

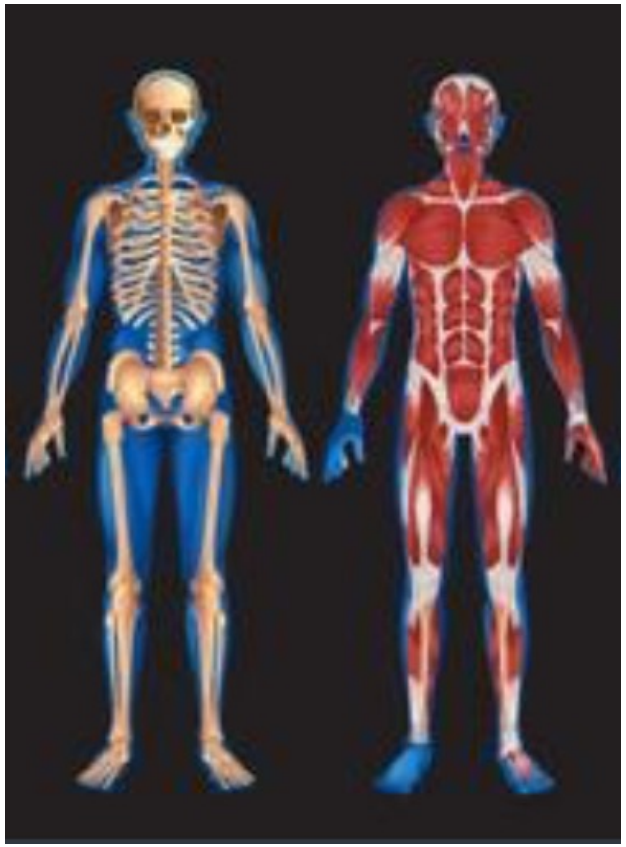
Bone and cartilage cells are normally restricted to the skeleton.

But in **heterotopic ossification**, bone and cartilage cells form within soft connective tissues such as skeletal muscle

and become organized into extra-skeletal bone tissue,

effectively replacing the tissue where heterotopic bone forms.

ACVR1 mutations in FOP induce a coordinated loss of tissue identity and maintenance and promote the acquisition of a new identity.



skeleton muscle

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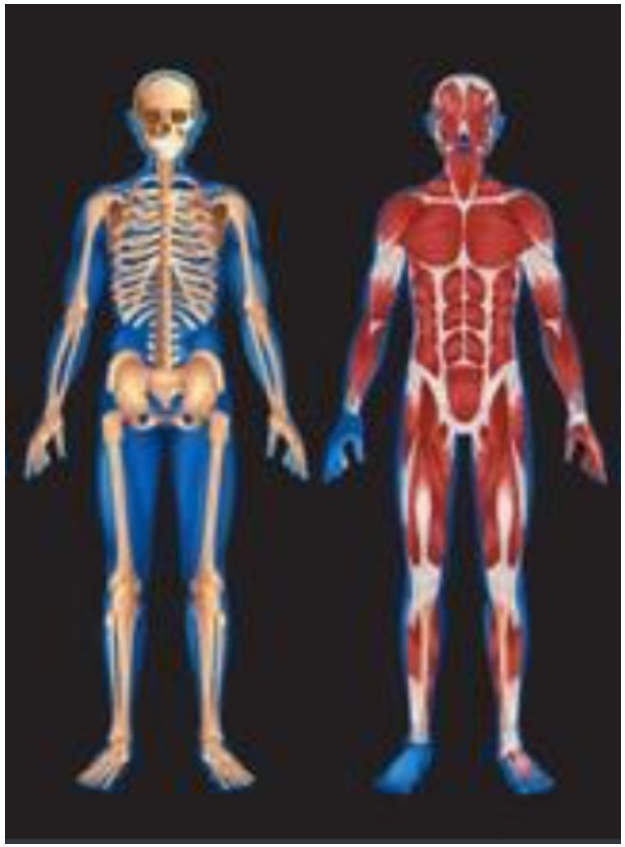
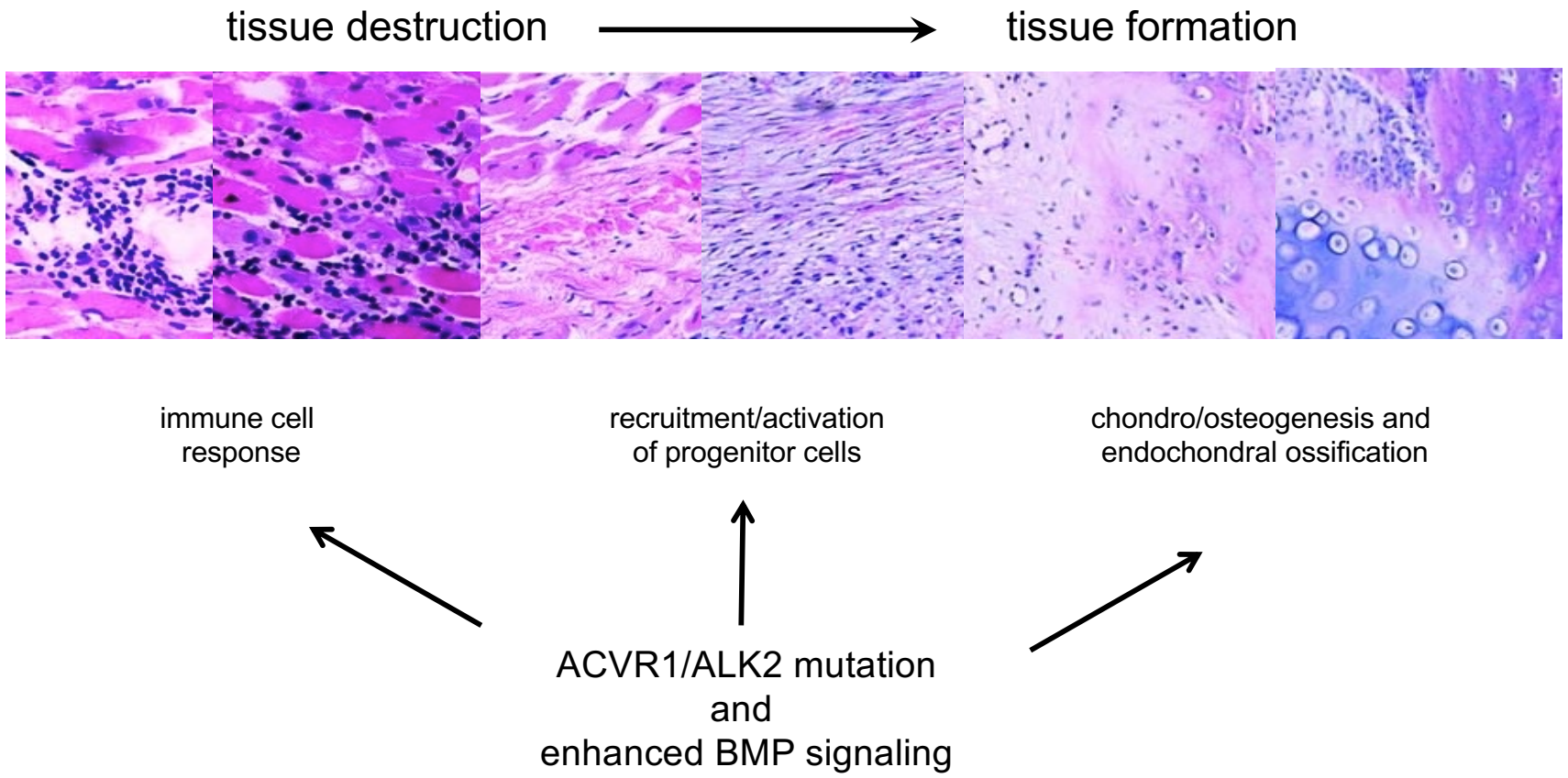


skeleton muscle

# Investigating the impact of the FOP ACVR1 mutation at multiple steps of the disease process.



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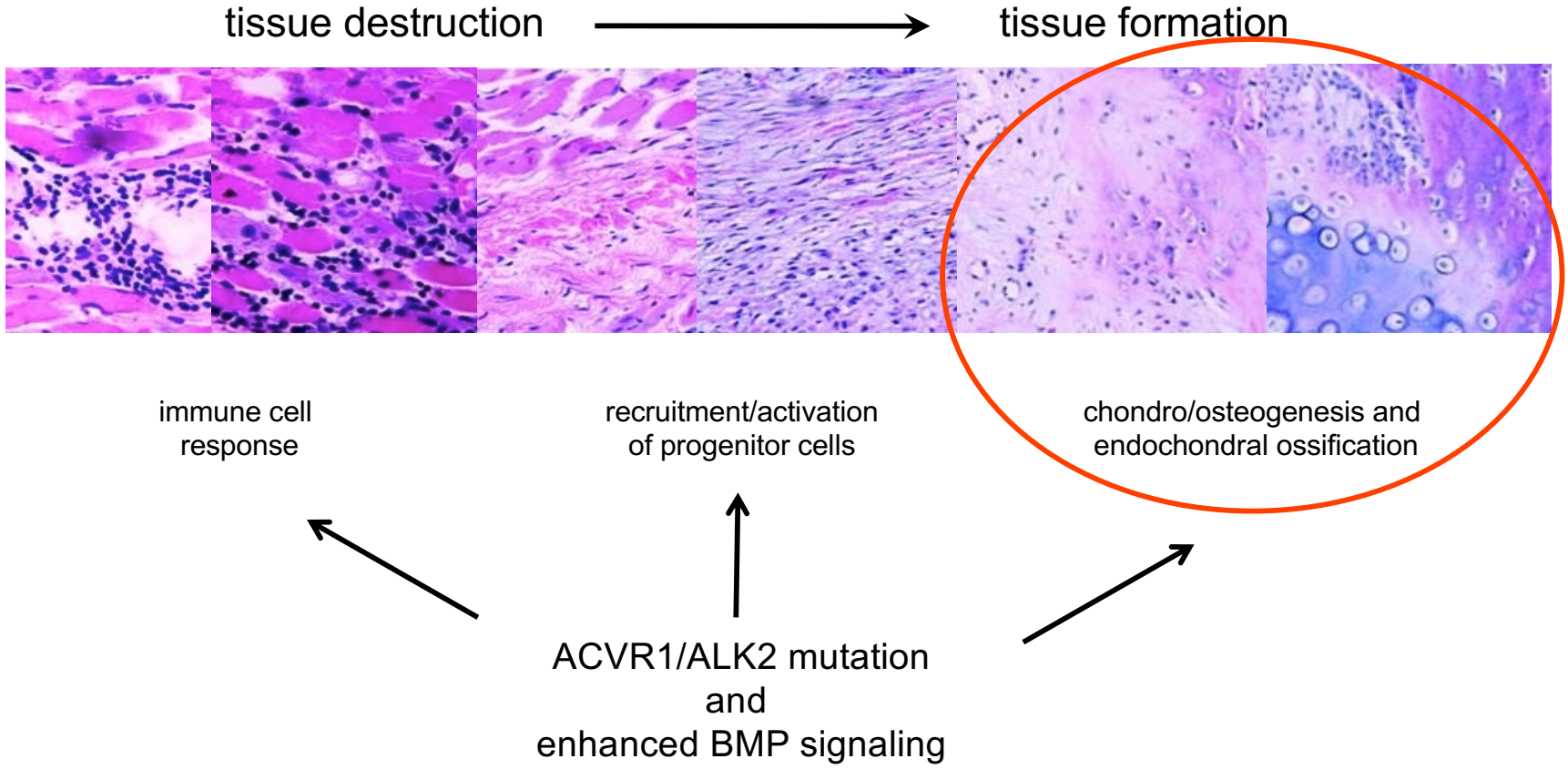

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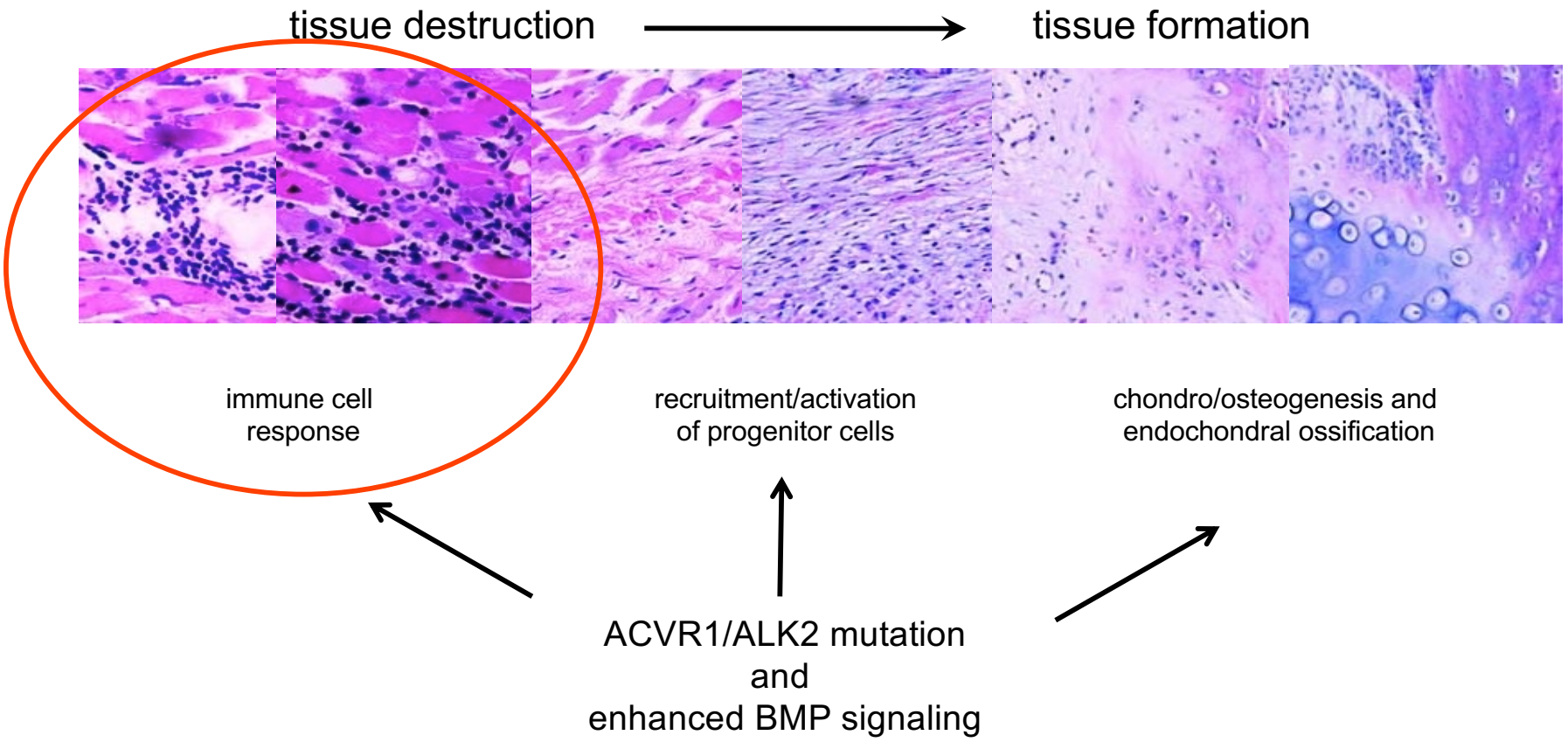
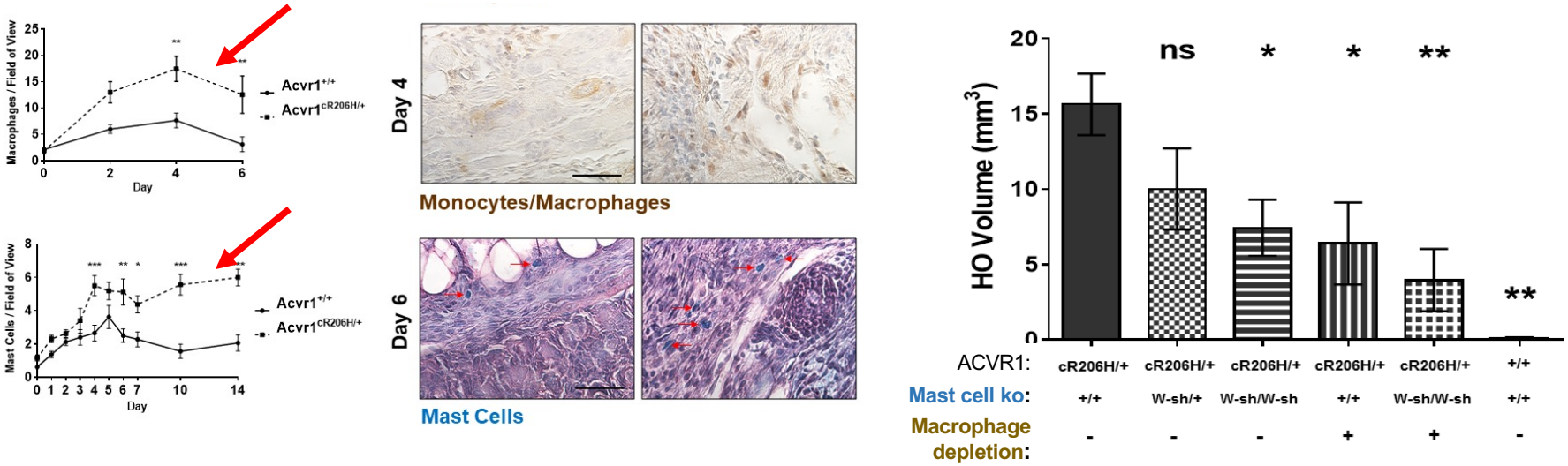


# Immune cell numbers are increased and persist in *Acvr1*$^{R206H/+}$ tissue post-injury




- Mast cell and macrophage depletion impair HO formation
- Expression of inflammatory cytokines is altered

Convente et al., 2018 *JBMR*

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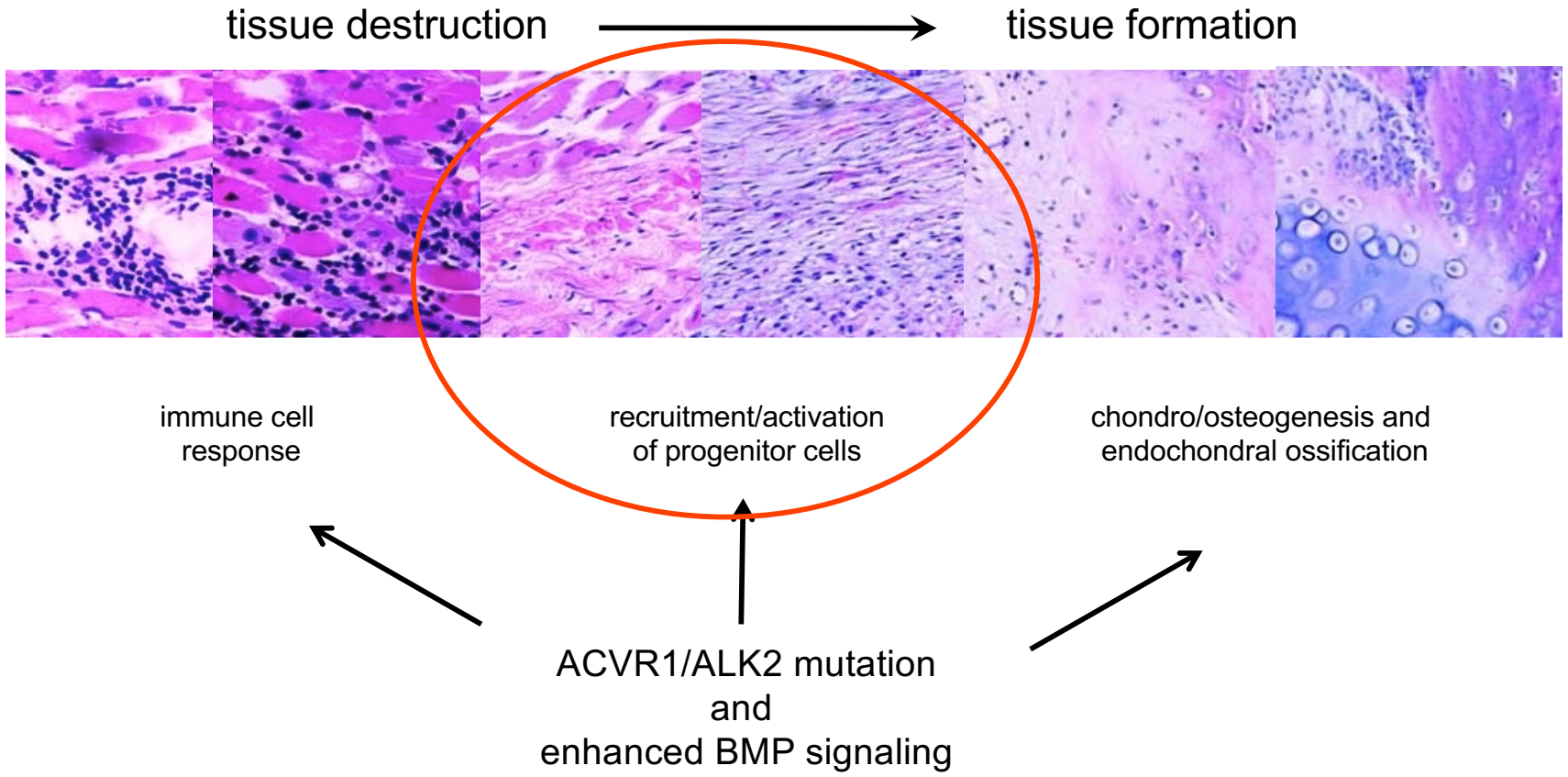
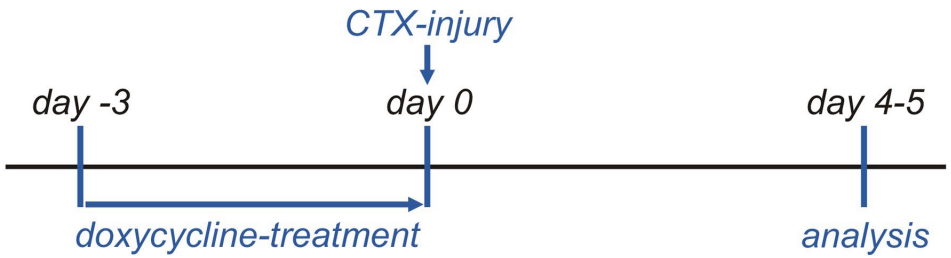
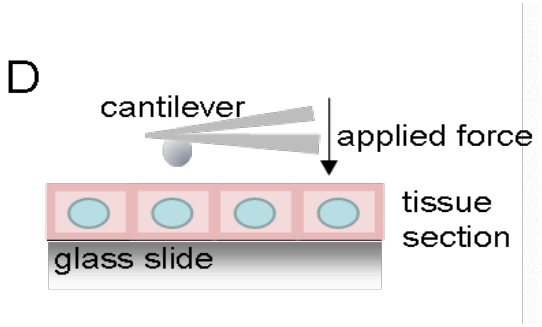
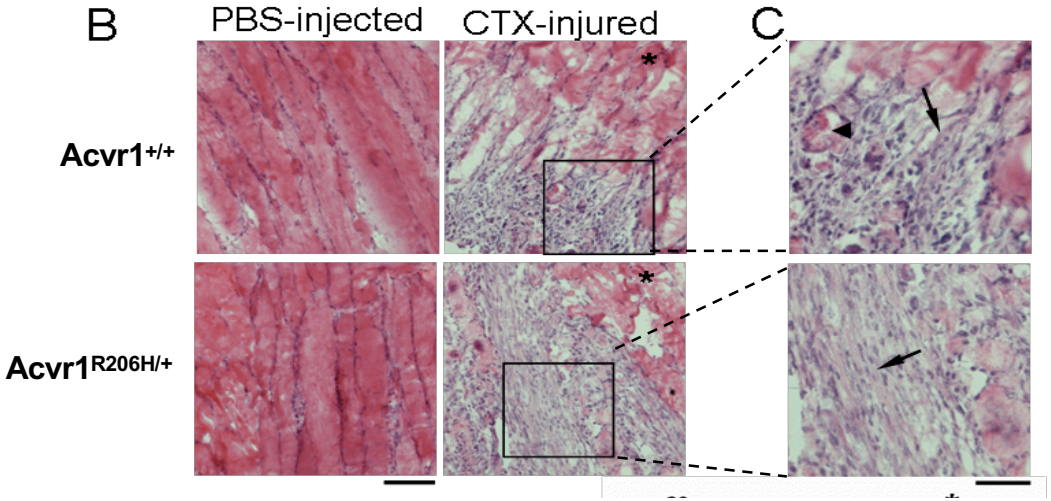
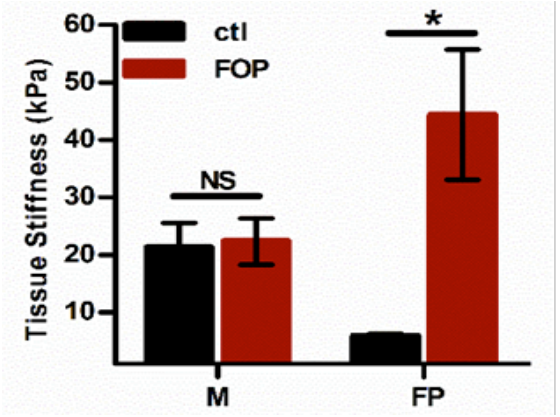


# Fibroproliferative tissue stiffness is increased and ECM is altered in response to skeletal muscle injury in *Acvr1*$^{R206H/+}$ mice



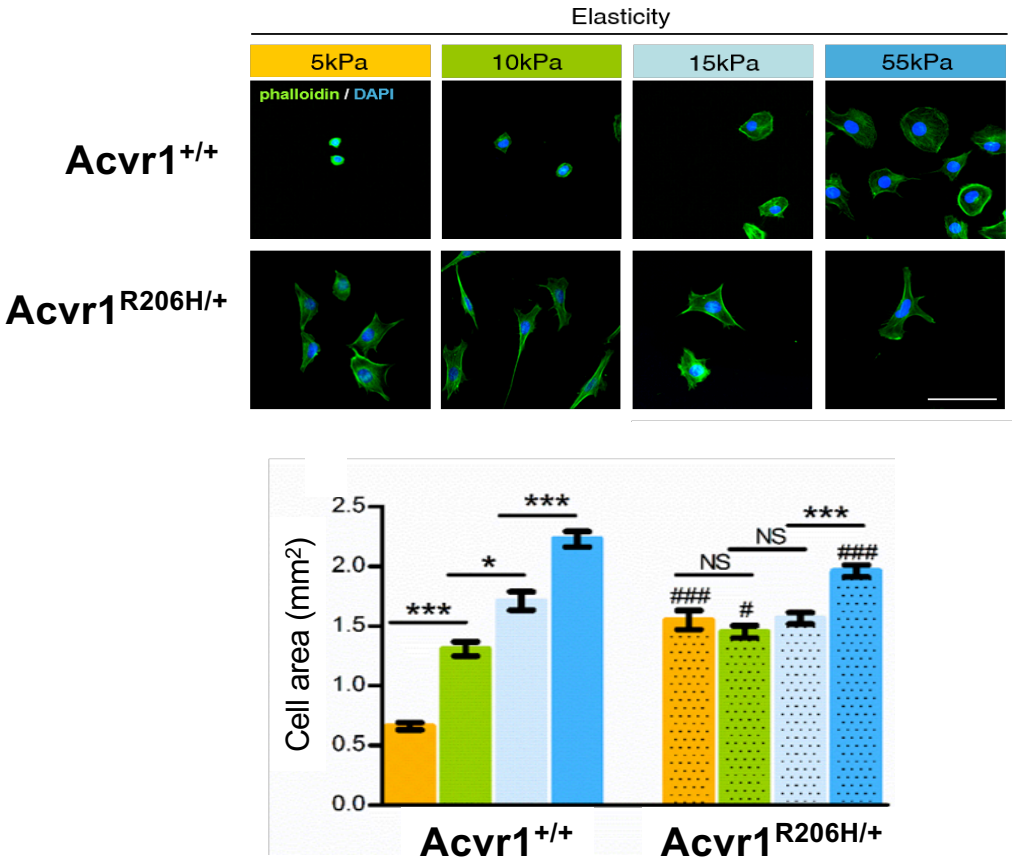




Haupt et al., 2019 *Mol Biol Cell*

# Mesenchymal cell response to their tissue microenvironment:
# - *Acvr1*$^{R206H/+}$ cells misinterpret substrate stiffness




MEFs; basal media

Haupt et al., 2019 *Mol Biol Cell*

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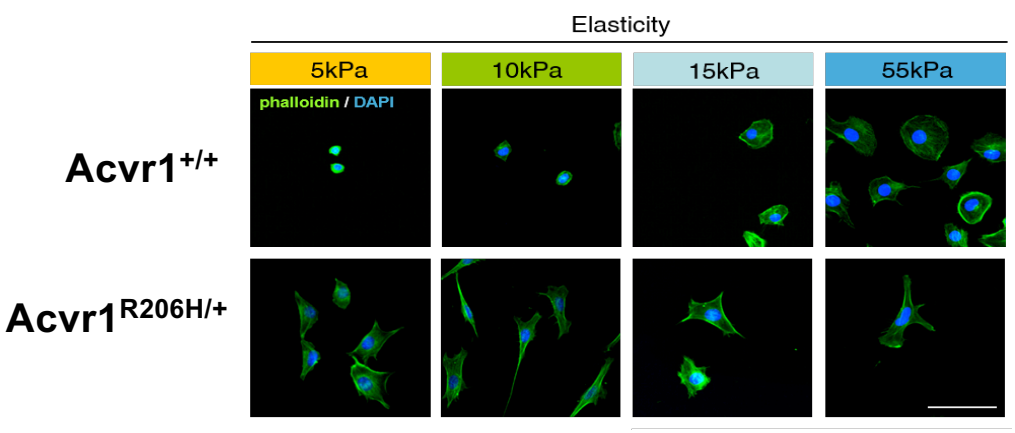
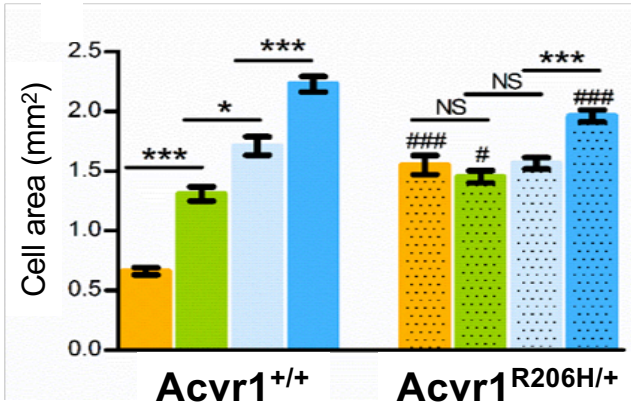



- *Acvr1*$^{R206H/+}$ cells misinterpret substrate stiffness

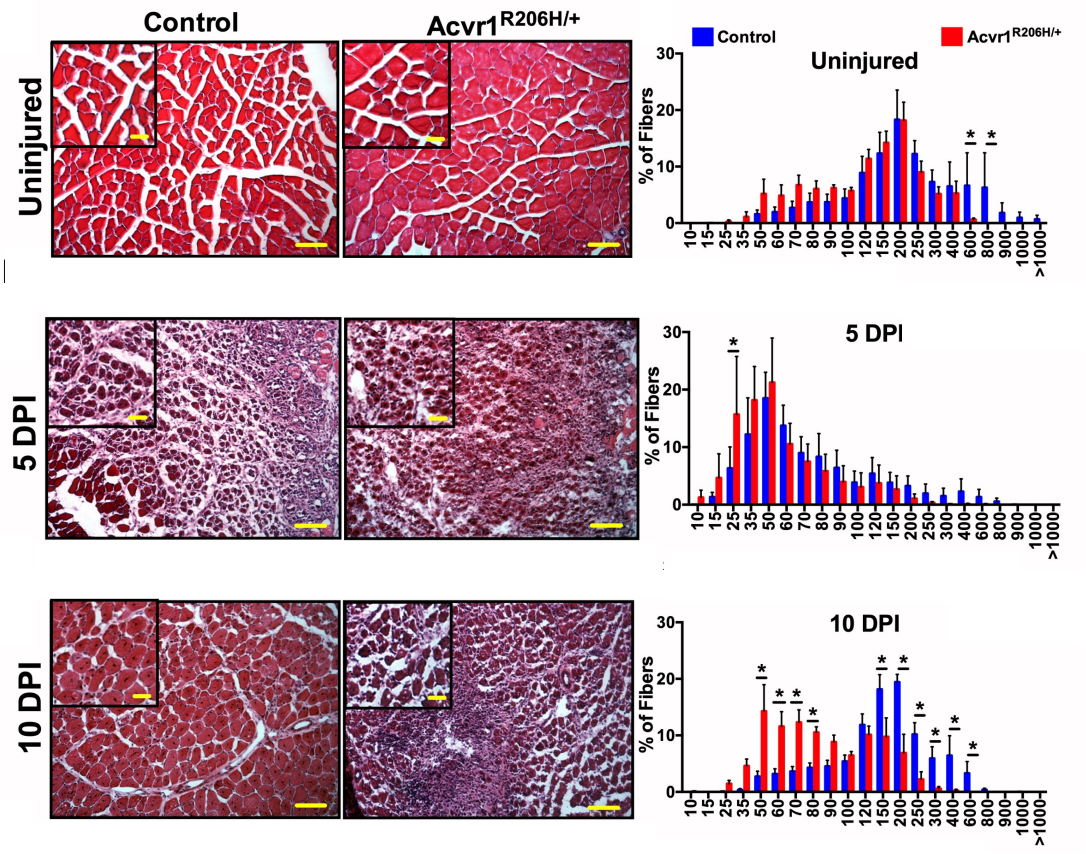
- *Acvr1*$^{R206H/+}$ cells are poised for osteogenesis
  - with increased expression of chondro/osteogenic genes in stiff environments

- *Acvr1*$^{R206H/+}$ cells show increased signaling through mechanosensing pathways, including
  - Rho/ROCK signaling
  - YAP/TAZ signaling

MEFs; basal media

Haupt et al., 2019 *Mol Biol Cell*

# *Acvr1*$^{R206H/+}$ skeletal muscle does not properly repair after injury




Stanley, Tichy et al., 2022 *npj Regen Med*

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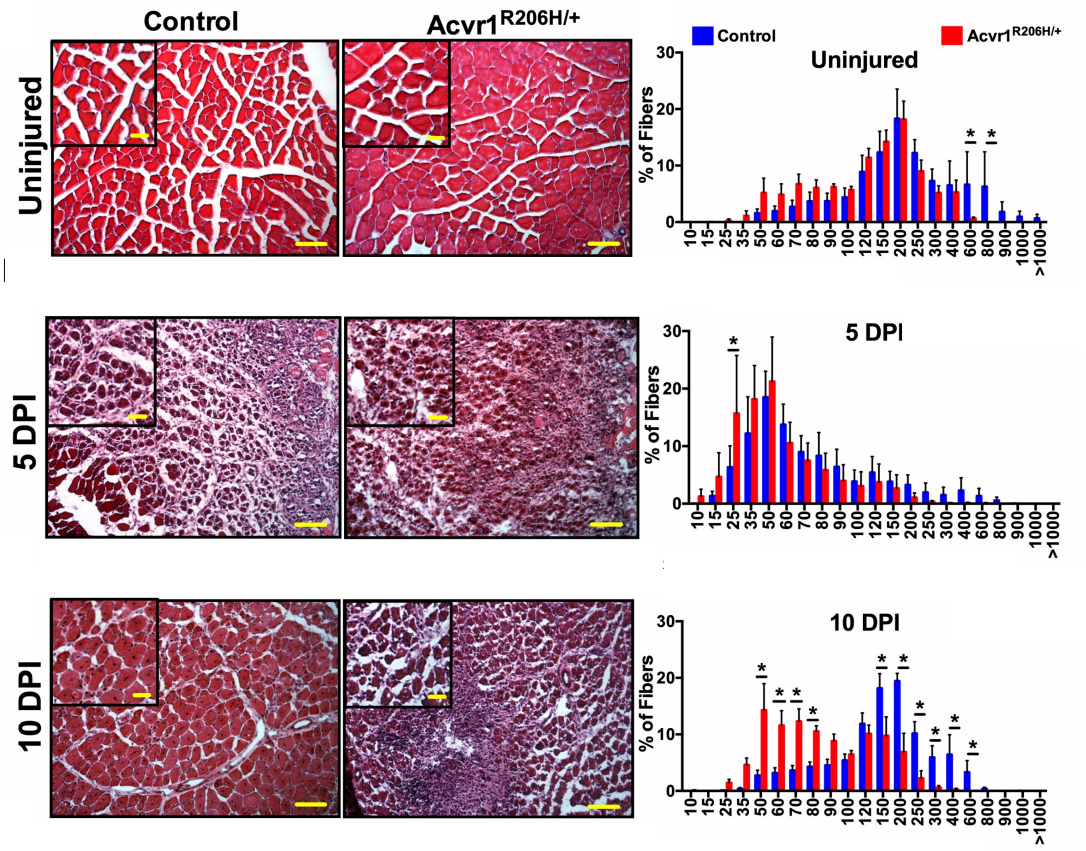
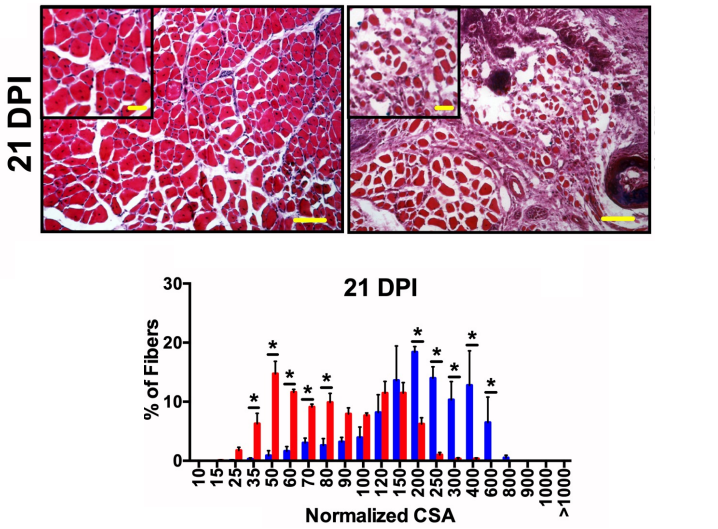
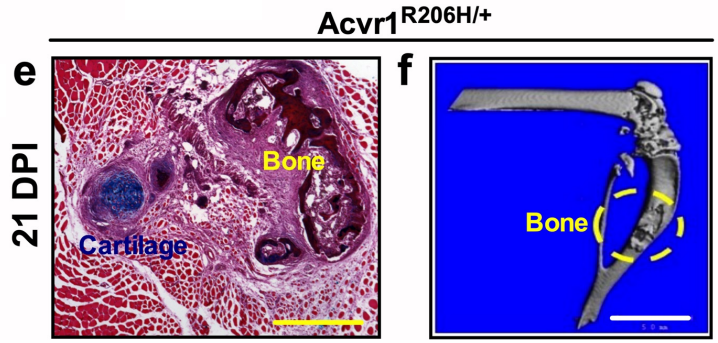


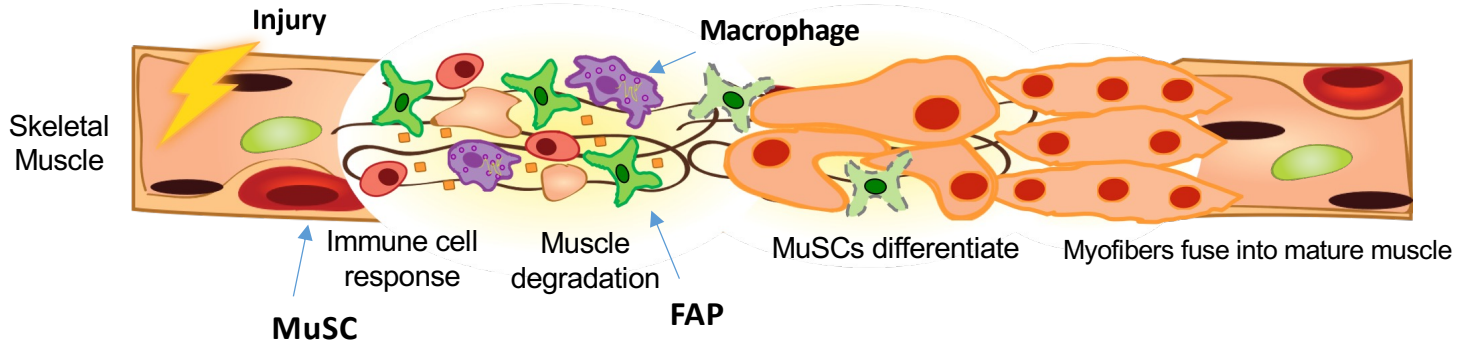

Stanley, Tichy et al., 2022 *npj Regen Med*

# *S*keletal muscle repair after injury – stem cell interactions

In response to skeletal muscle injury

- mesenchymal cells (fibro/adipogenic progenitor cells; FAPs) activate muscle stem cells (MuSCs) and then undergo apoptosis and return to quiescence

- muscle stem cells differentiate and fuse to form new fibers and repair muscle tissue




**Mesenchymal stem cells** have chondrogenic and osteogenic ability

**Muscle stem cells** do not differentiate to cartilage and bone cells.

Stanley et al., 2019 *Encyl Tissue Engineering*

# *Acvr1*$^{R206H/+}$ skeletal muscle does not properly repair after injury

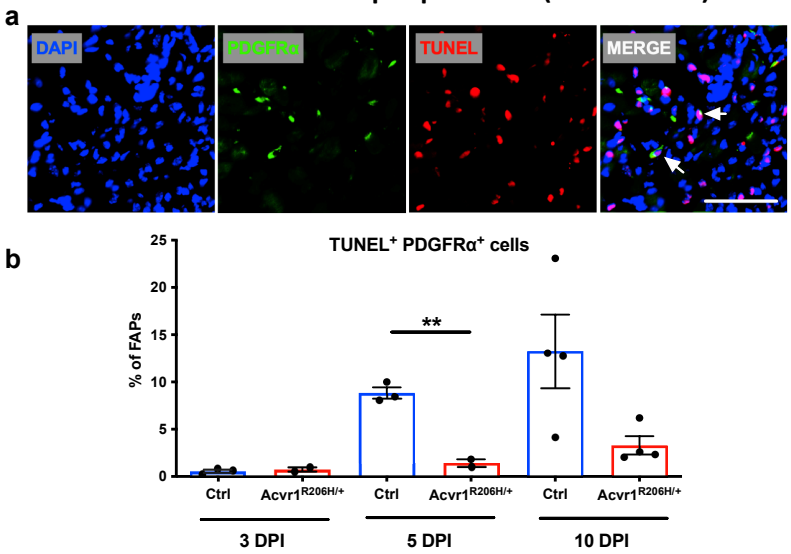
MuSCs are not lost to increased apoptosis (cell death)

MuSCs do not transdifferentiate to bone/cartilage cells

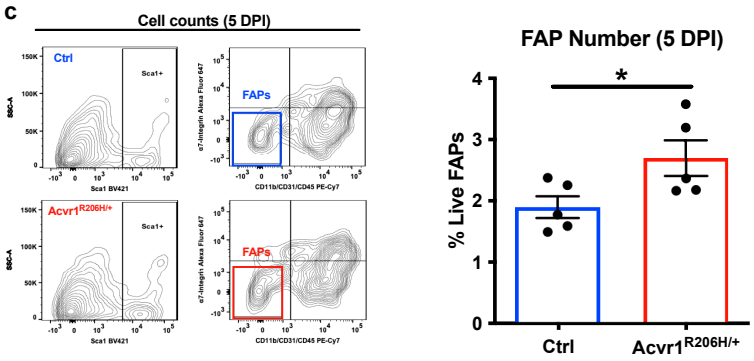
No differences in FAP or MuSC proliferation compared to controls

# However, *Acvr1*$^{R206H/+}$ FAPs fail to decline during regeneration

Reduced FAP apoptosis (TUNEL+)

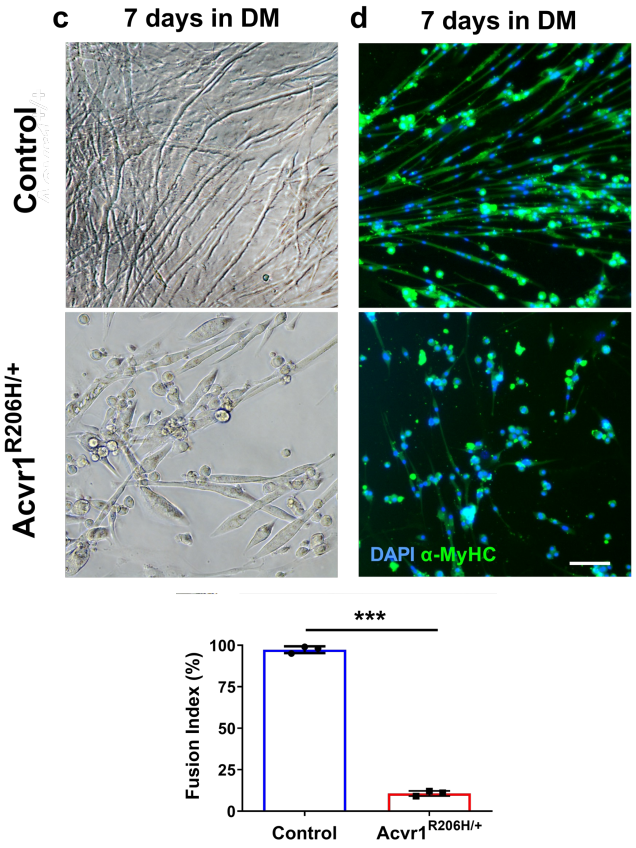



Increased FAP numbers

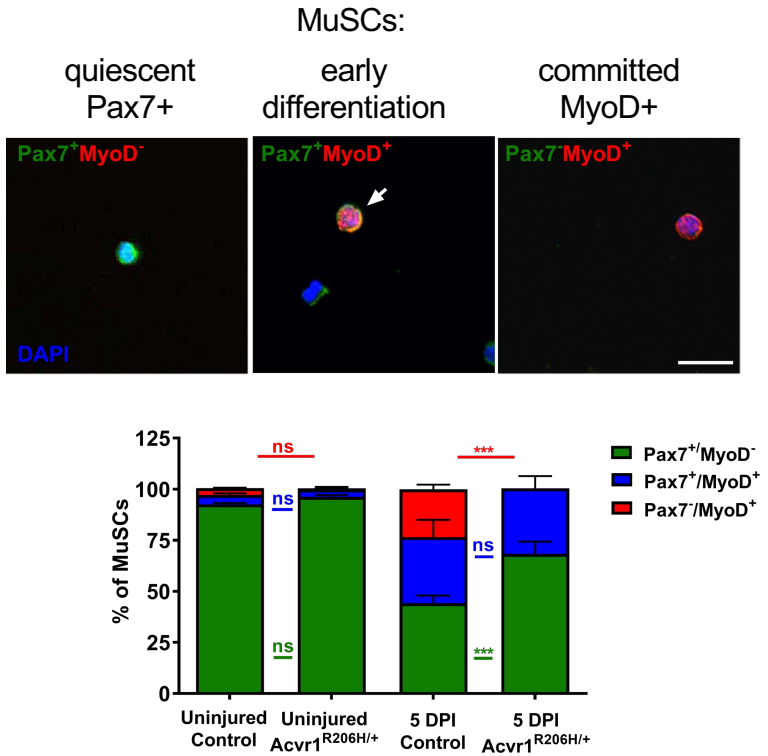



Stanley, Tichy et al., 2022 *npj Regen Med*

# Impaired differentiation of *Acvr1*$^{R206H/+}$ MuSCs



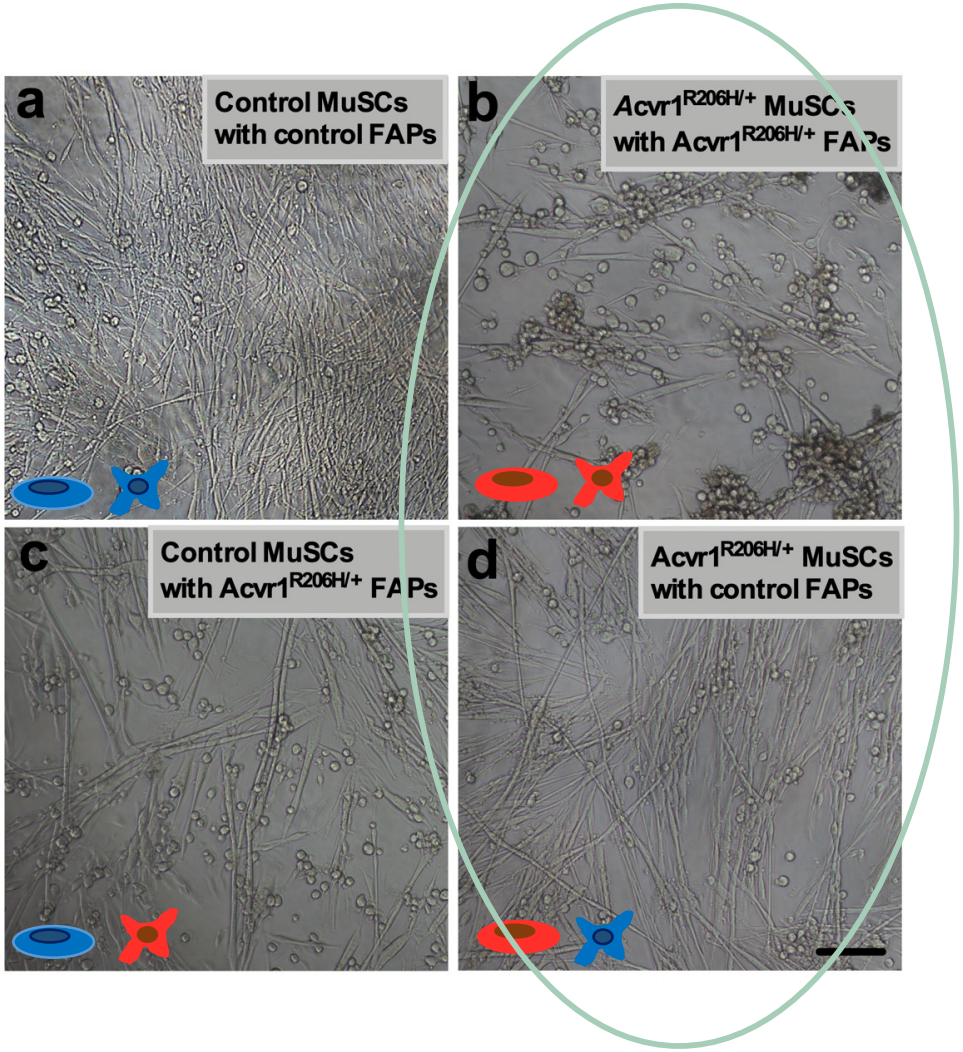

Reduced myofiber fusion to multi-nucleate cells *in vitro*

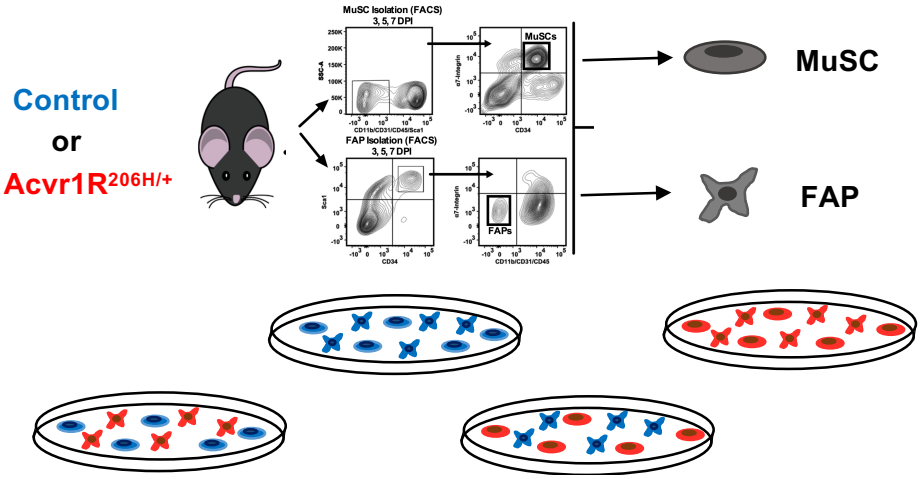


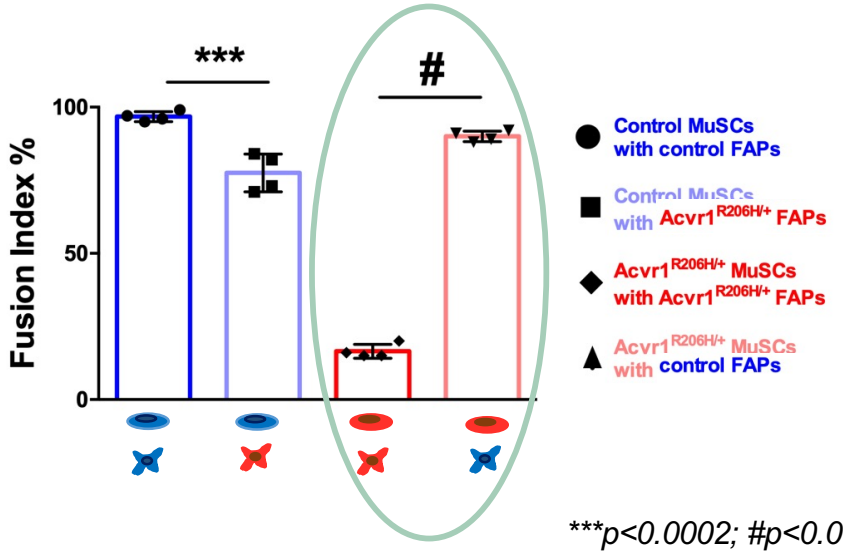

Reduced differentiation to committed (MyoD+) MuSCs *in vivo*

Stanley, Tichy et al., 2022 *npj Regen Med*

# Myogenesis in *Acvr1*$^{R206H/+}$ MuSCs is rescued by control FAPs




***p<0.0002; #p<0.0001

# Heterotopic ossification

Heterotopic ossification is a coordinated loss of tissue identity and acquisition of a new identity.

As the process of new bone formation begins within soft connective tissues such as skeletal muscles, the integrity of the muscle and its ability to repair is compromised.

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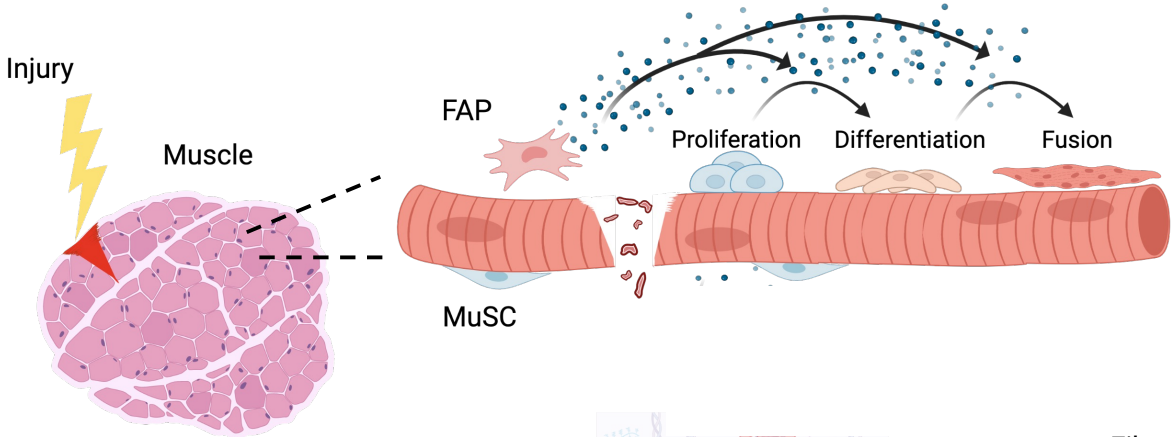
How do FAPs contribute to skeletal muscle repair after injury?

How is this process altered by $Acvr1^{R206H}$?

# Skeletal muscle repair after injury



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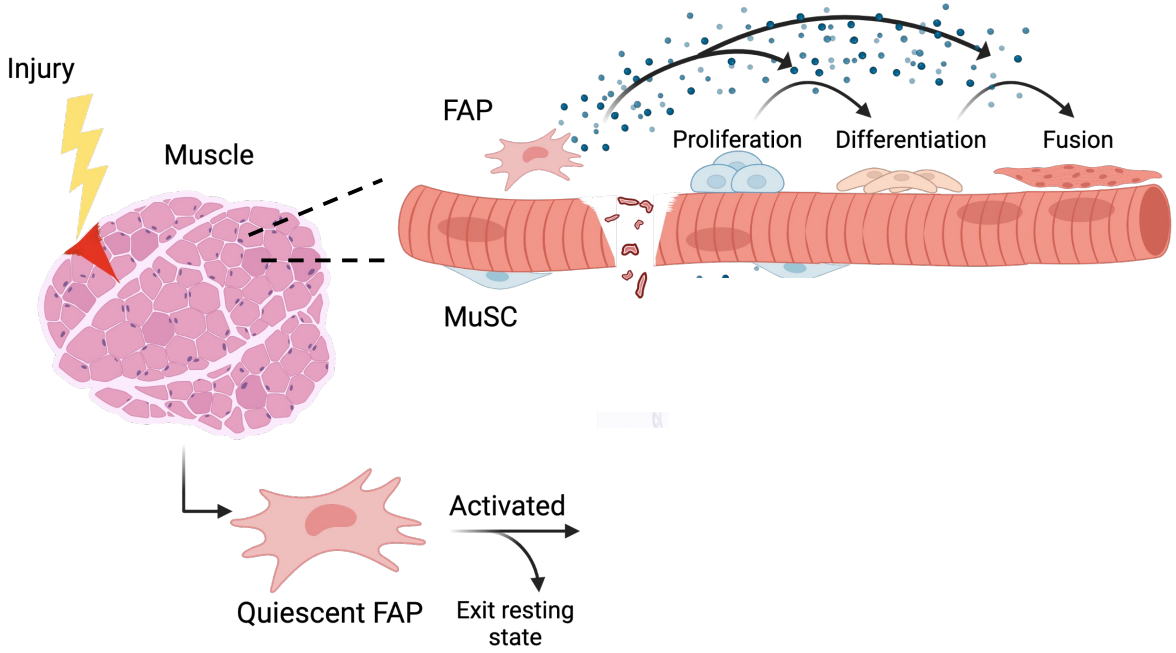

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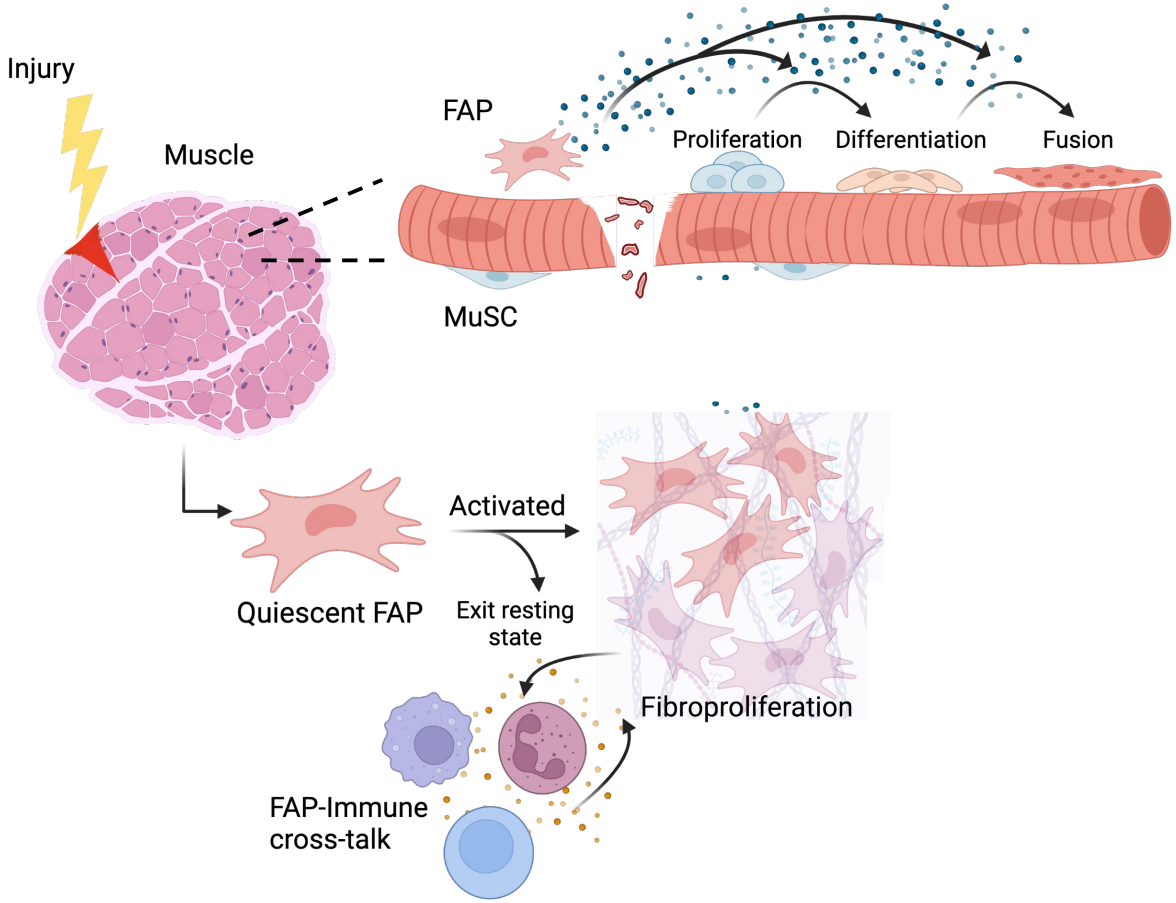

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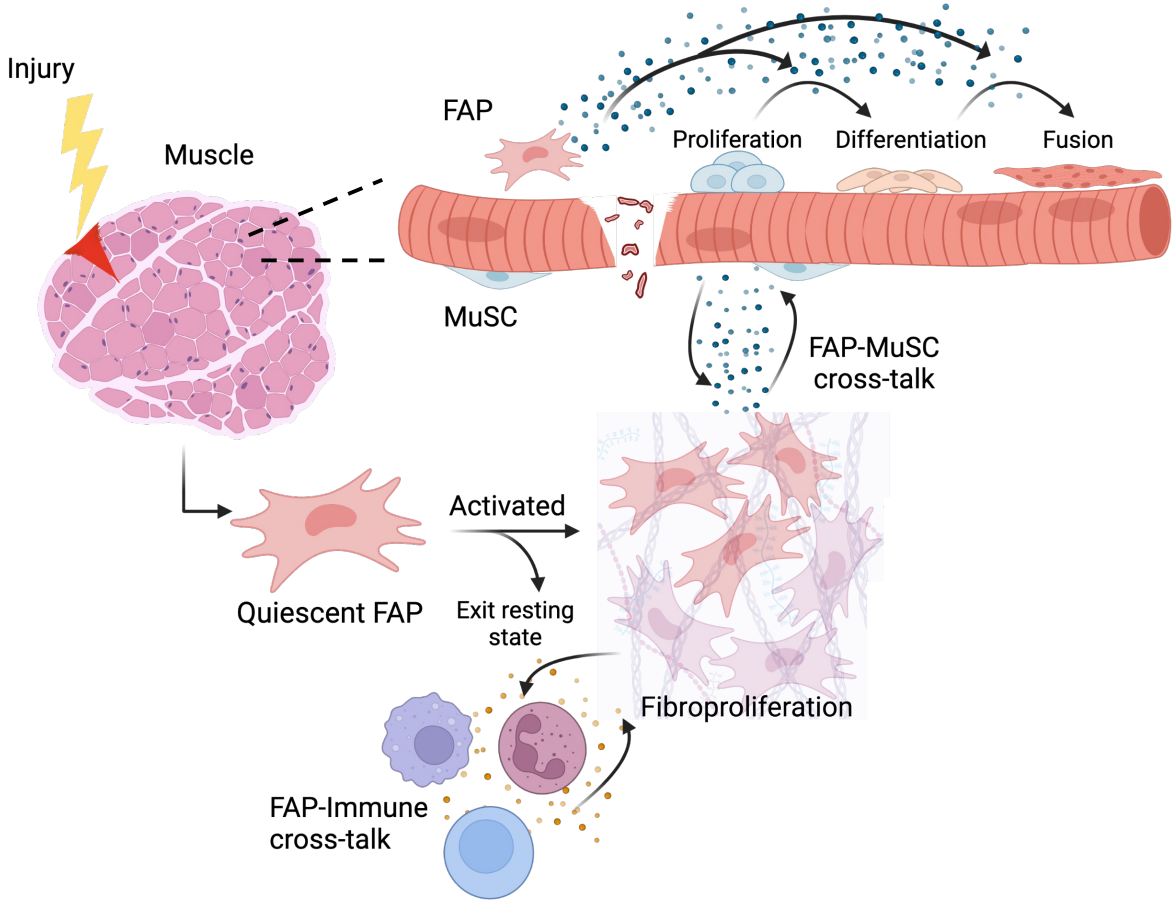
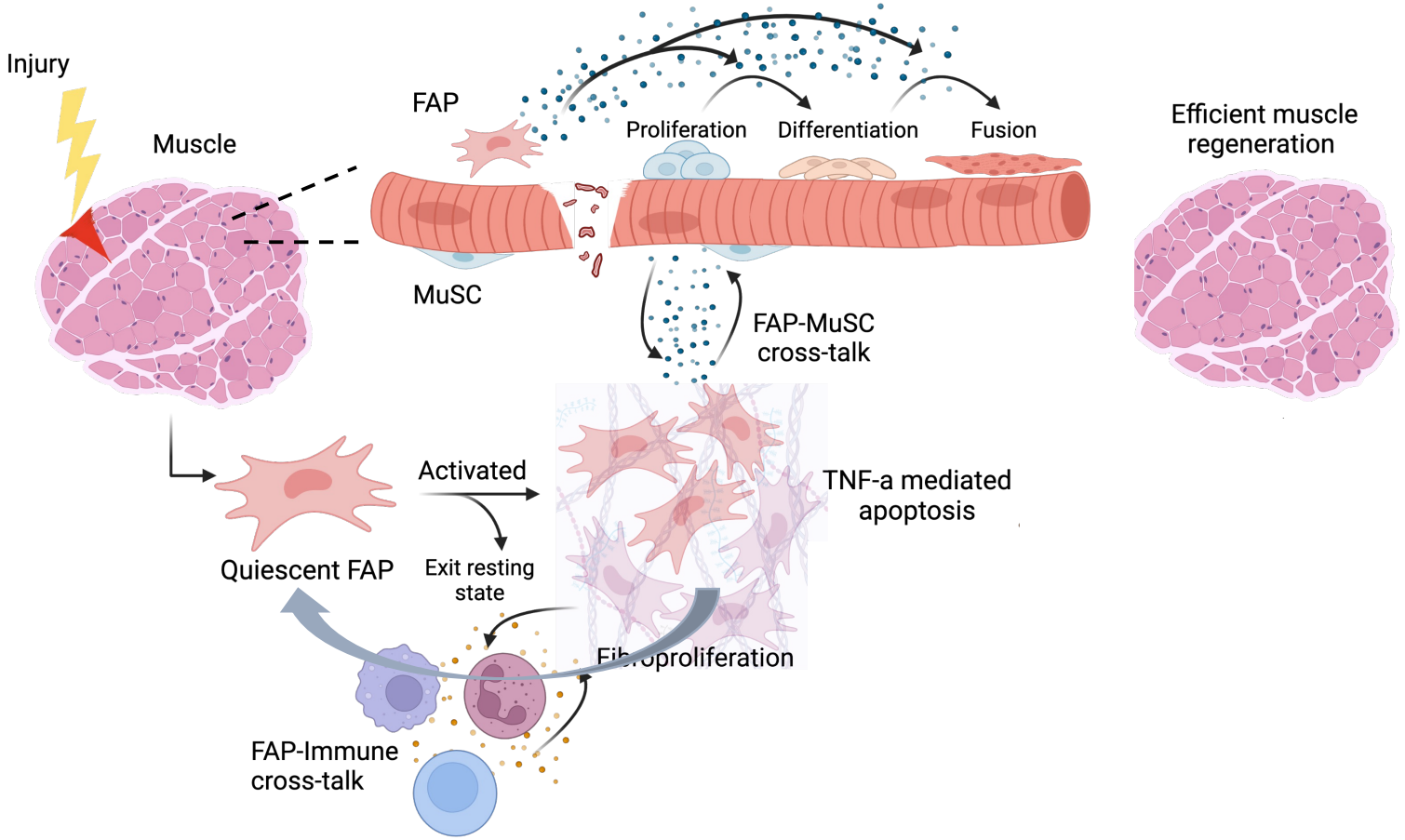
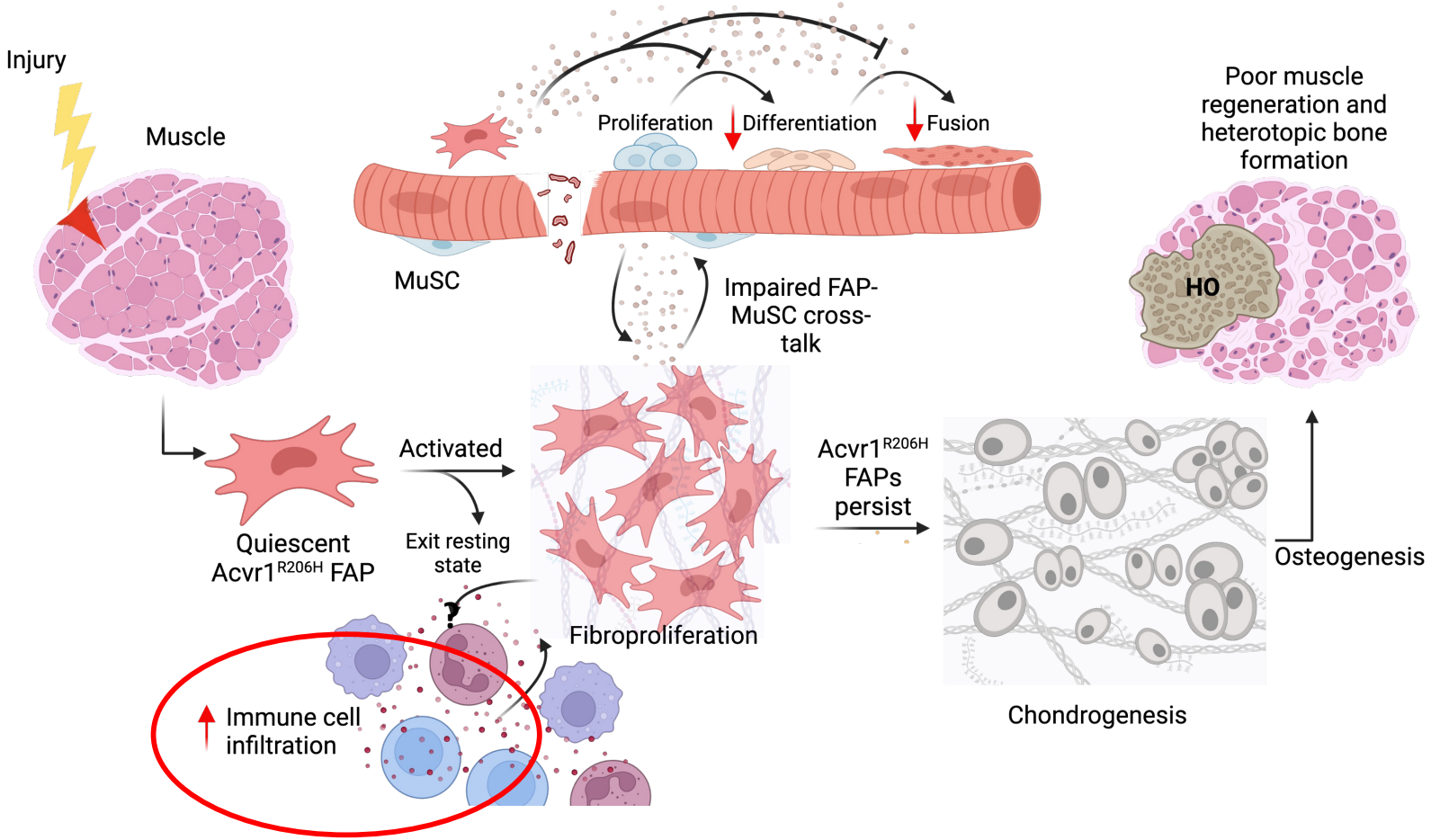


# *Skeletal muscle repair after injury*



# Skeletal muscle repair after injury in FOP




Stanley, A., Tichy, E.D., Kocan, J. et al. npj Regen Med 7, 5 (2022).
Convente, M. R. et al. 33(2), 269–282 J Bone Miner Res. (2018).

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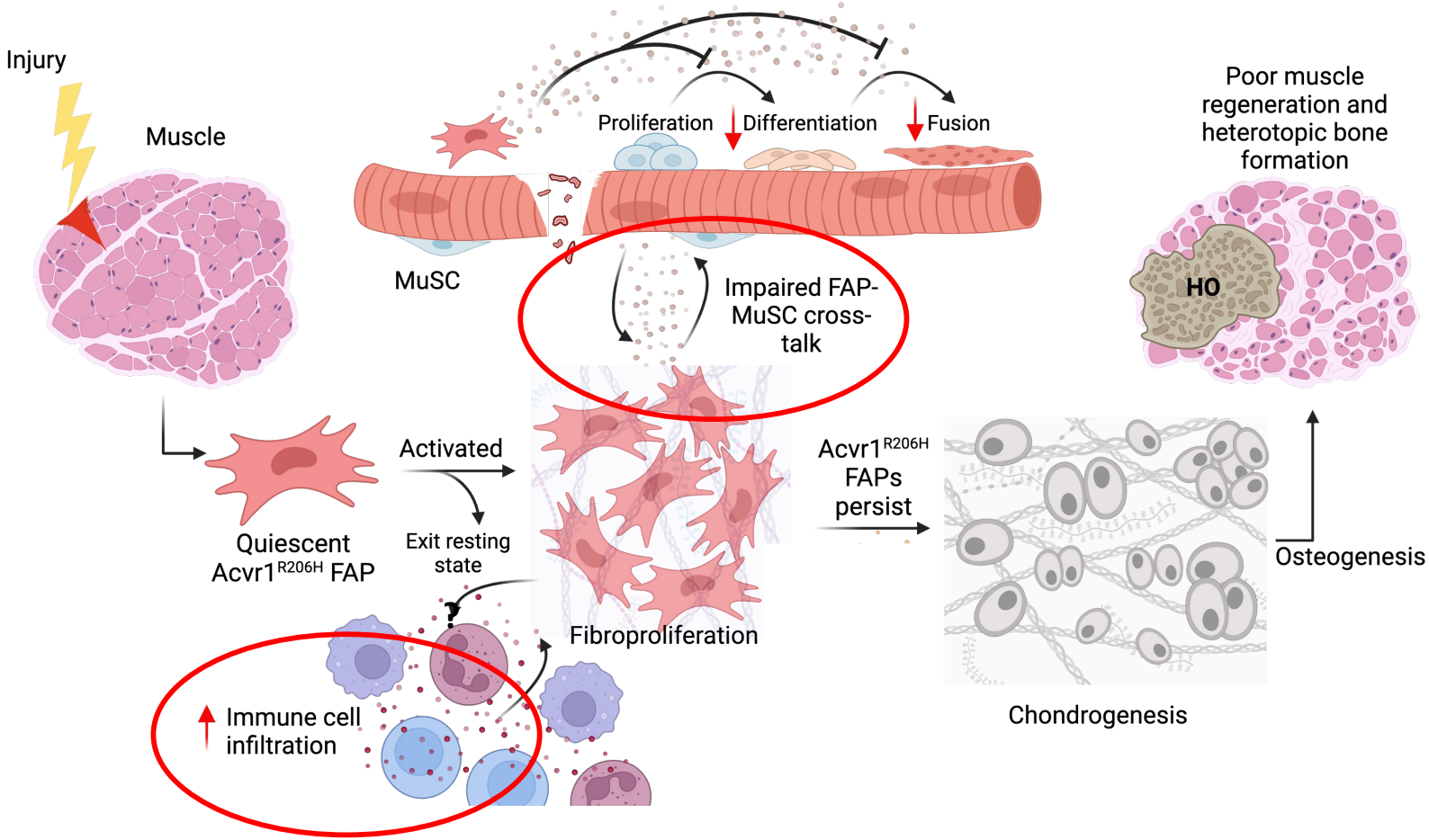


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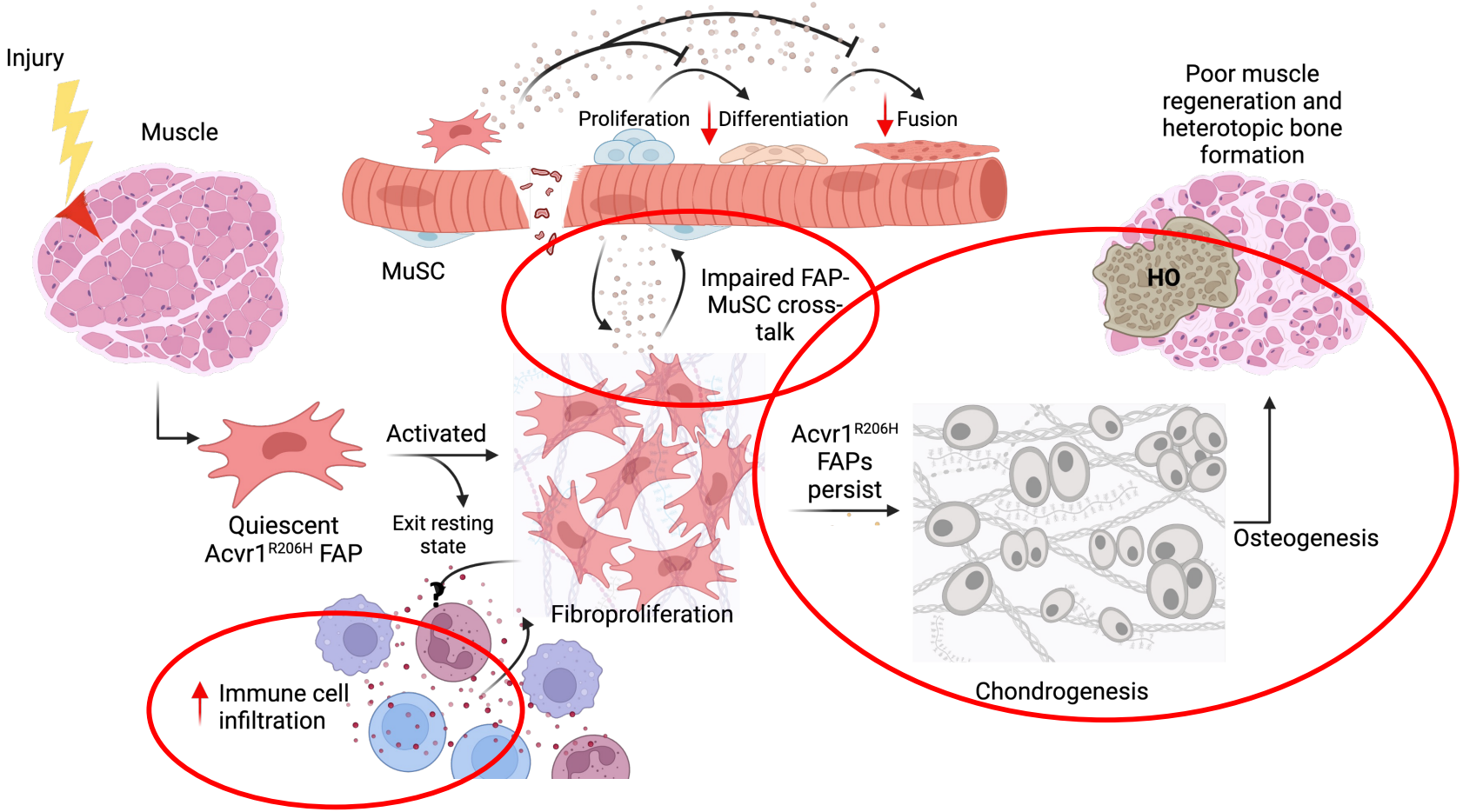


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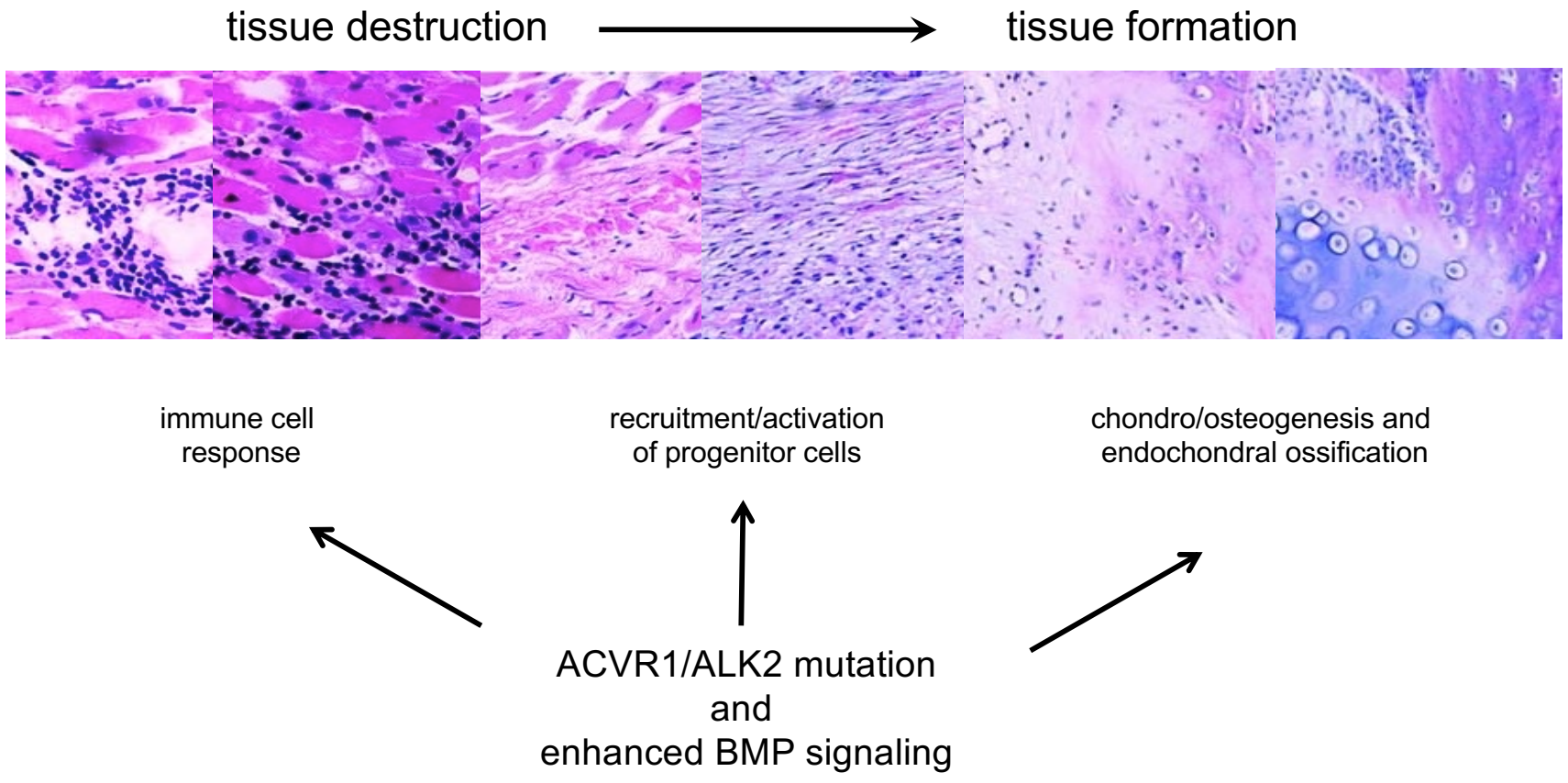
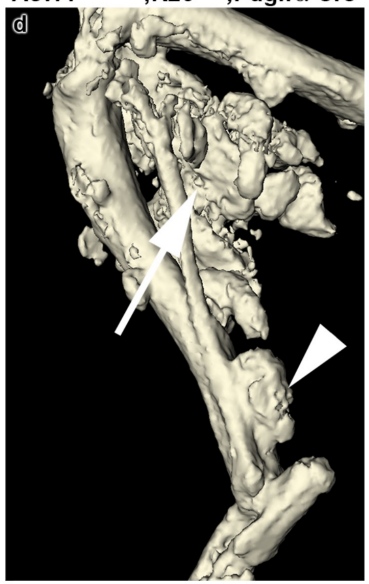
# Heterotopic ossification is a coordinated loss of tissue identity and acquisition of a new identity



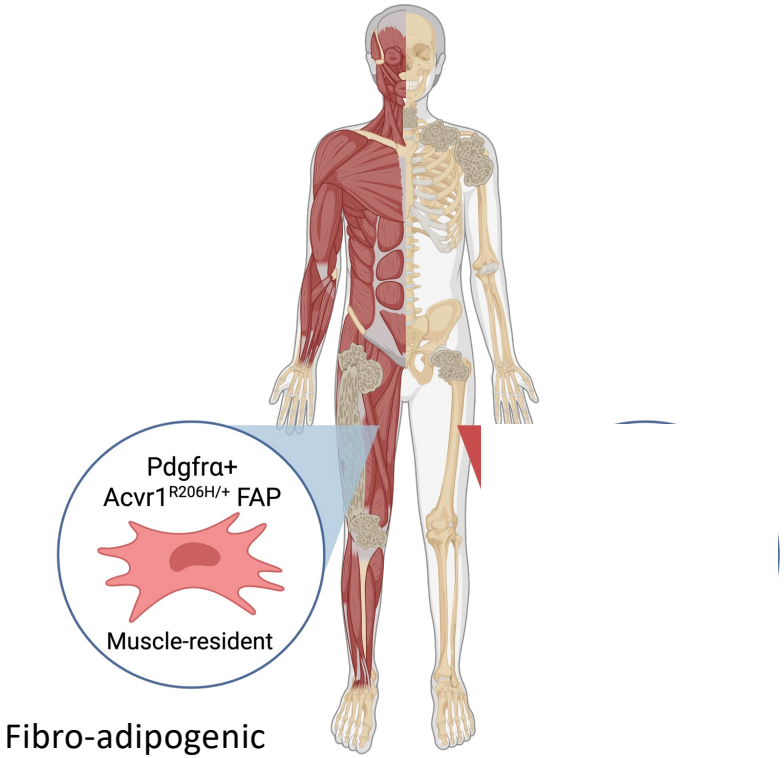
# *Acvr1*$^{R206H}$ Pdgfr$\alpha$-lineage cells are HO progenitor cells

**Day 14 Post-Cardiotoxin**

***Acvr1***$^{tnR206H/+}$***;R26***$^{NG/+}$**;Pdgfr$\alpha$-Cre**




Lees-Shepard, J. B., et al. *Nature Communications.* 9, 471 (2018).




Fibro-adipogenic Progenitor

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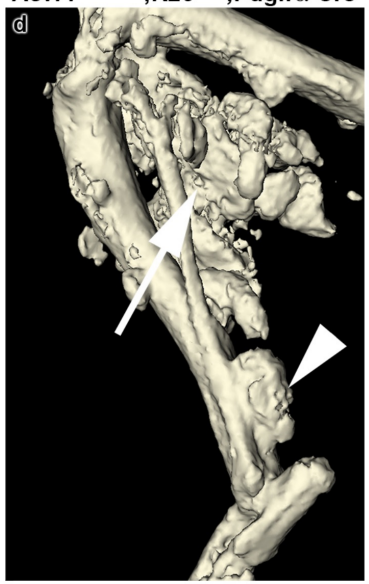


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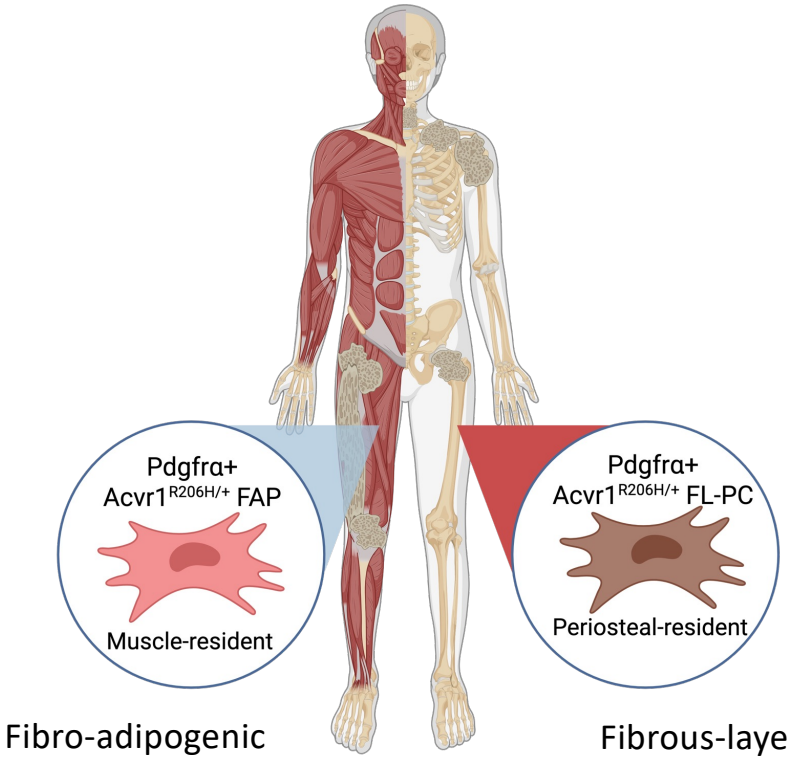


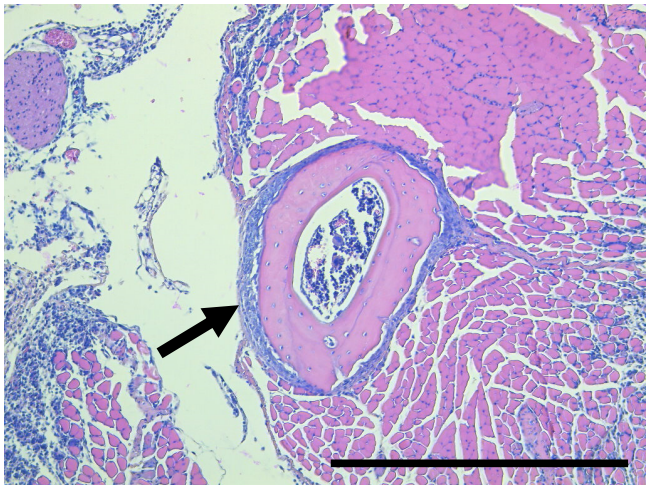

Fibro-adipogenic Progenitor

Fibrous-layer Periosteal Cell

# *Acvr1*$^{R206H}$ fibula periosteum expands and ossifies after muscle injury

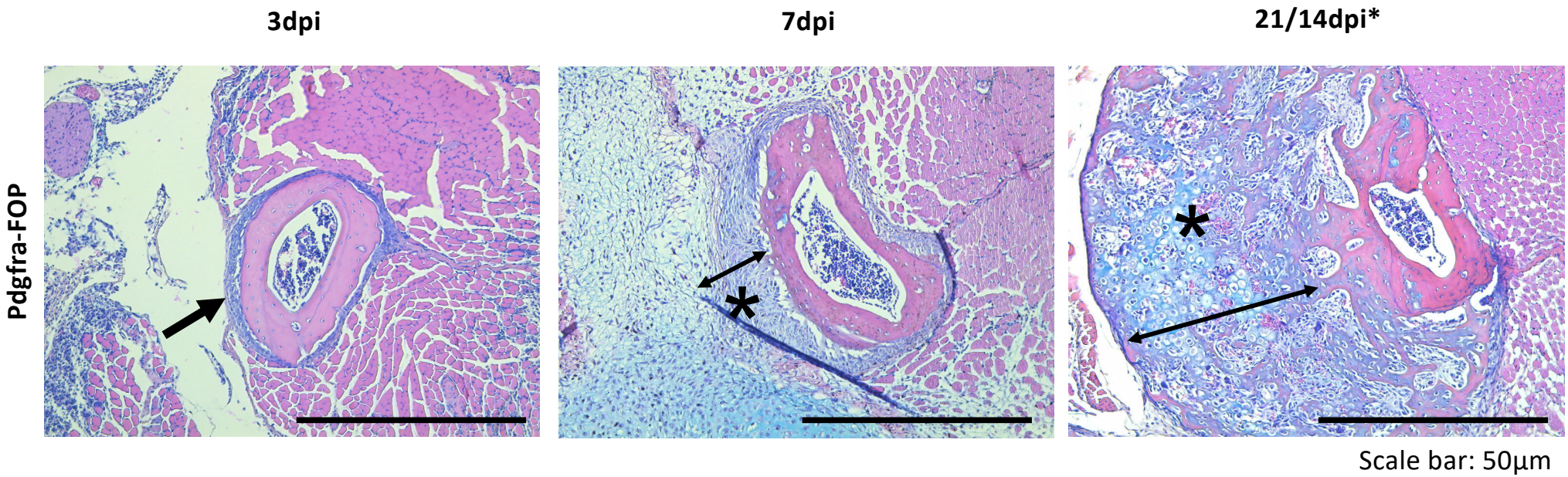
**3dpi**

**Pdgfra-FOP**



Scale bar: 50µm

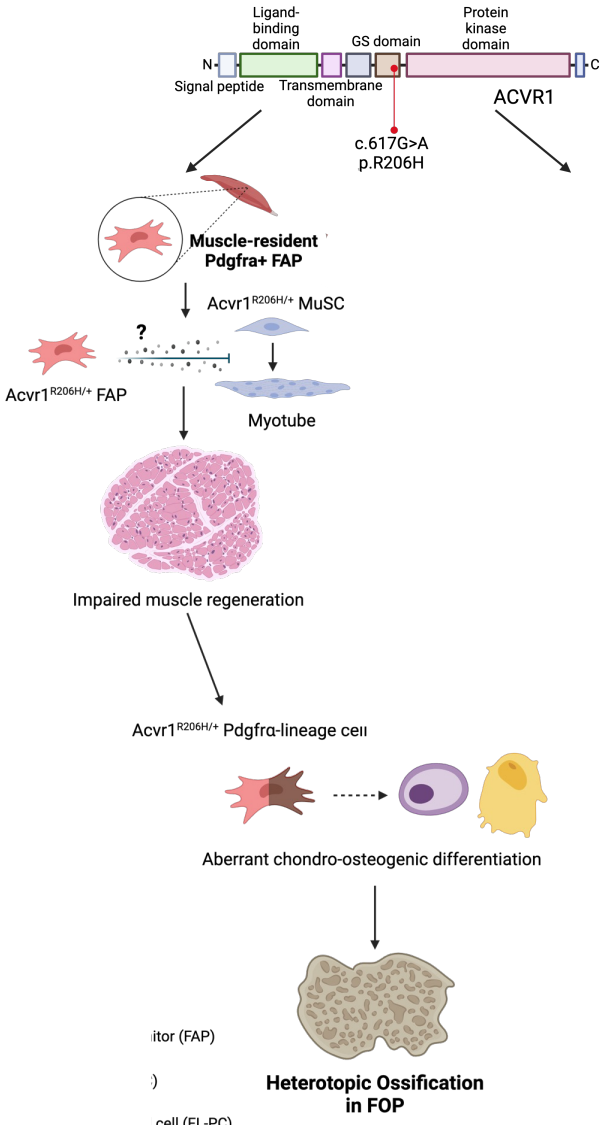
# *Acvr1*$^{R206H}$ fibula periosteum expands and ossifies after muscle injury

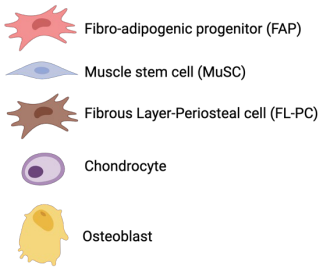


*Working Model –*

FOP progenitor cells for heterotopic ossification:

Pdgfra+ mesenchymal cells

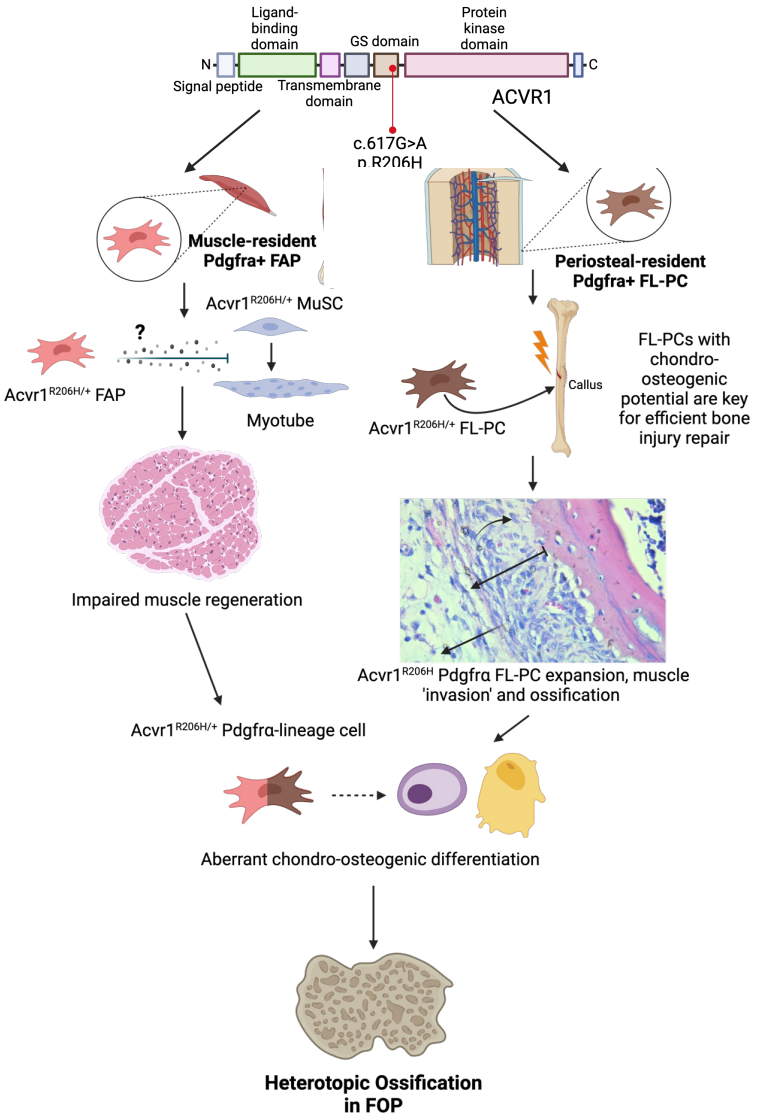


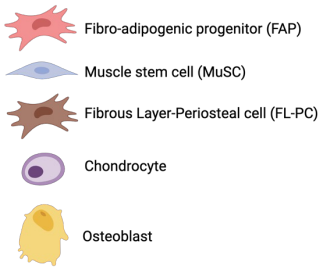



*Working Model –*

FOP progenitor cells for heterotopic ossification:

- multiple populations and sources of Pdgfra+ mesenchymal cells



## Key points

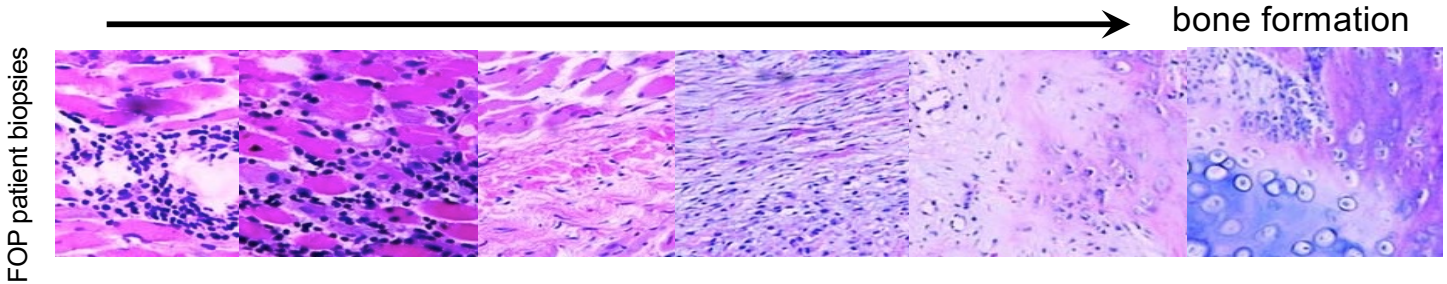
Heterotopic ossification is a coordinated loss of tissue identity and acquisition of a new identity.

In FOP, ACVR1 is no longer constrained by the usual molecular mechanisms that regulate signaling activity by the BMP receptor complex.

Increased BMP pathway signaling

- poises mesenchymal stem cells toward chondro/osteogenesis
- alters the responses of other cells (such as muscle stem cells) within the tissue microenvironment where HO will form

Increased BMP signaling through mutant ACVR1 alters the interactions among cells and changes the identity of the vulnerable tissue.



## Key points

Heterotopic ossification is a coordinated loss of tissue identity and acquisition of a new identity.

BMP signaling pathways interact with other cellular pathways, including biomechanical signaling pathways, and are key mediators that regulate tissue formation, repair, and maintenance.

In the context of HO, it is important not to consider progenitor cells in isolation, but as part of an integrated and coordinated system of cells and their environment.

Our ongoing work is examining the dynamic effects of biomechanical signaling as a key mediator of this system including their downstream regulation of chromatin structure and gene expression.

Fred Kaplan, Mona Al Mukaddam
and their clinical team

**Robyn Allen***

Niambi Brewer*

**Salin Chakkalakal**

**Michael Convente***

**Andria Culbert***

Nadine Grossmann

**Julia Haupt***

Vitali Lounev

**Loreilys Mejias Rivera**

**Doug Roberts**

Tzipora Schein

**Alexandra Stanley***

Will Towler*

Jeffrey Xi*

**Meiqi Xu**

Jianing Xu

Deyu Zhang

*former lab member

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FOP Italia – and many others!
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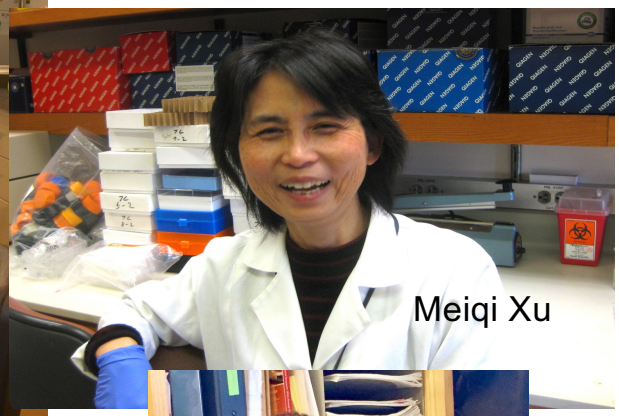
Cali and Weldon Family Endowments
FOP Families and Friends

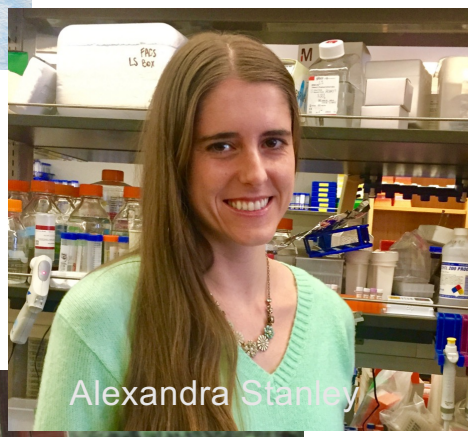

Salin Chakkalakal


Michael Convente


Andria Culbert


Meiqi Xu


Qi Shen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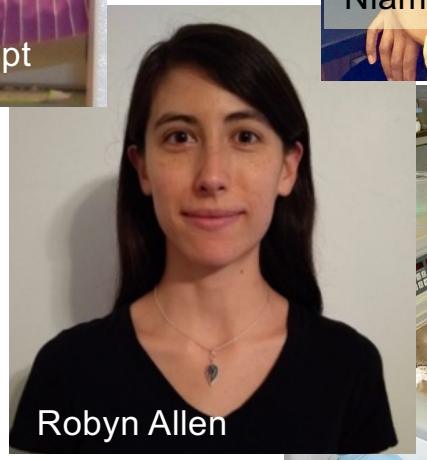
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