

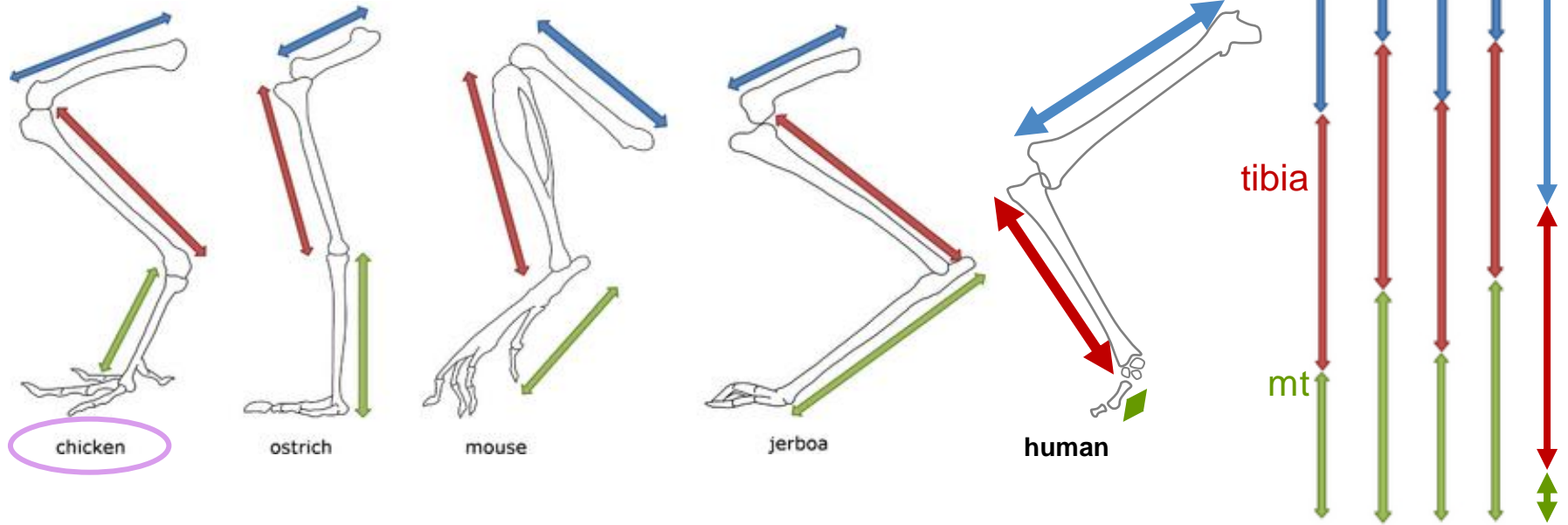
# *Regulation of Joint Development by Mechanical Factors*

OARSI, 2015



Andrew Pitsillides  
Professor of Skeletal Dynamics  
*Skeletal Biology@RVC*  
*Royal Veterinary College*

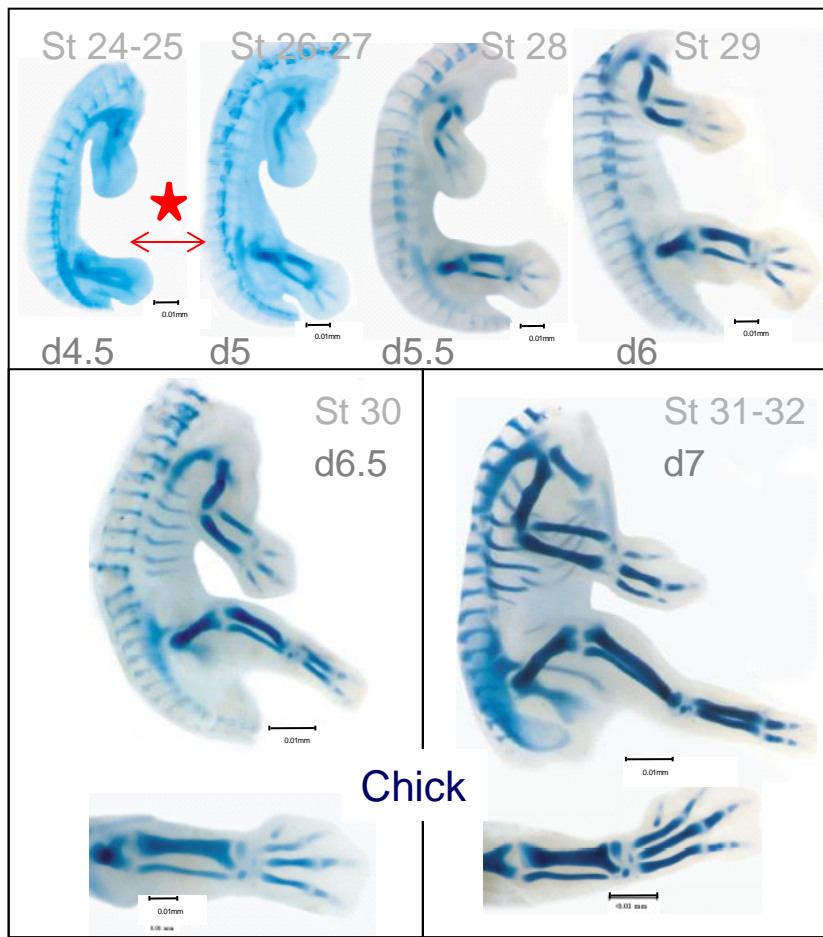
# Limb proportionality



- Variation in limb proportions
- Joint shape and position?
- Huge impact for locomotion
- Emerge during development
- Role of mechanical input?

RVC

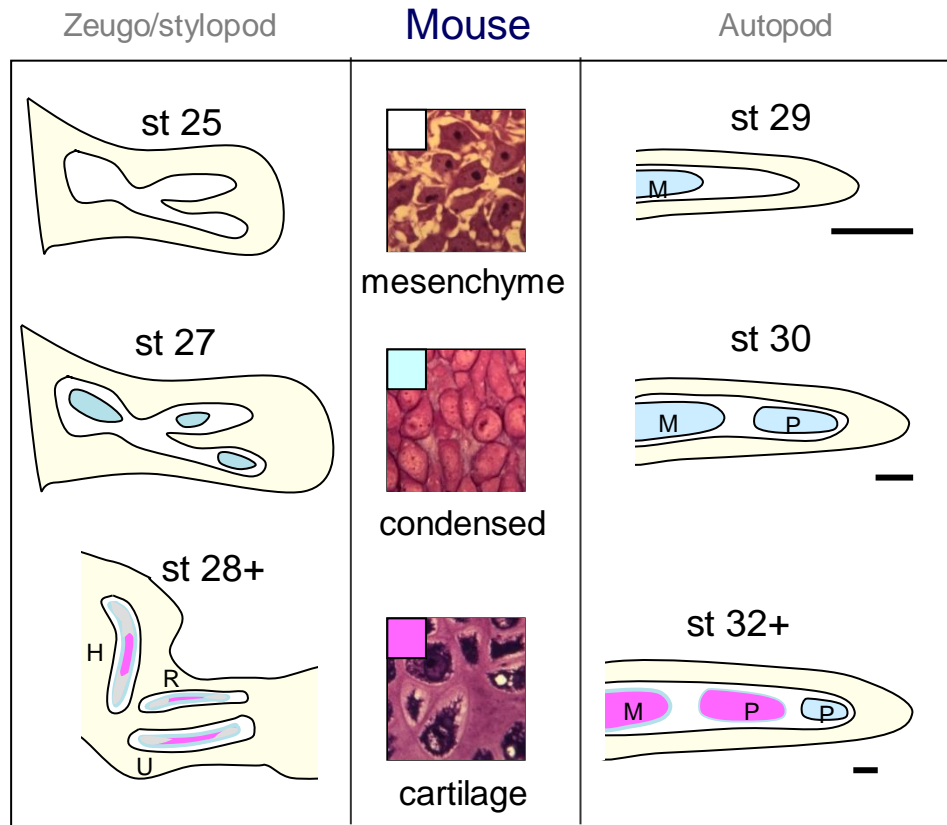
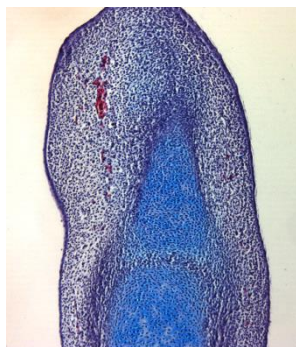
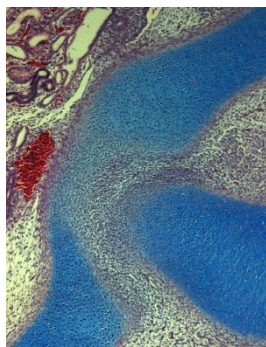
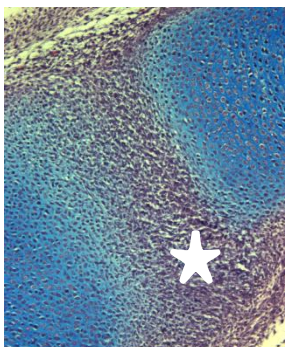




Knee St 32

Hip St 32

DIP St 35



**P-D/ Interzone**

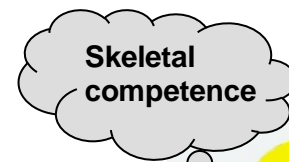
**Huge expansion**

**Cartilage growth:**

- Proliferation
- Maturation
- Hypertrophy

**Move very early – day 5**

*Hamburger, Balaban, 1963*

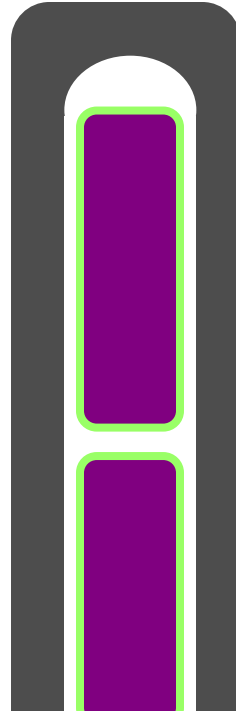
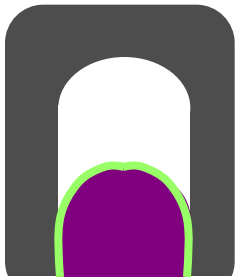


**Movement**

Skeletal elements distinct from the outset

### Joint morphogenesis

Patterning normal without movement



### Joint 'cavitation'



in ovo paralysis

*How does movement contribute to joint formation?*

*Does movement contribute to limb proportionality?*

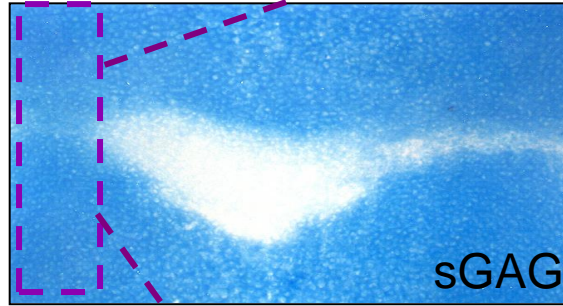
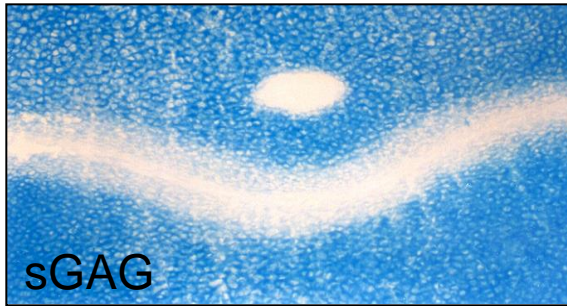
*Mechanism of cavitation*



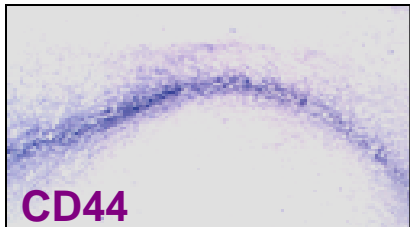
# Movement drives joint-formation and limits default chondrocyte fate

Control

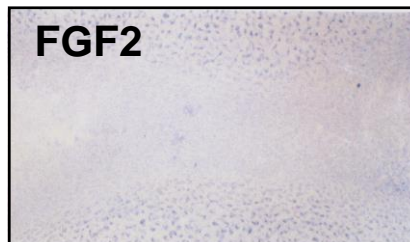
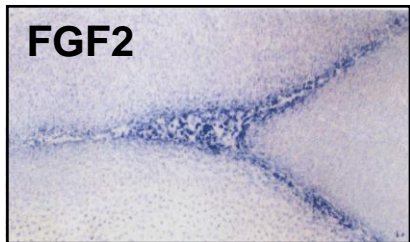
Immobilised



Stages 36-39  
TT joints

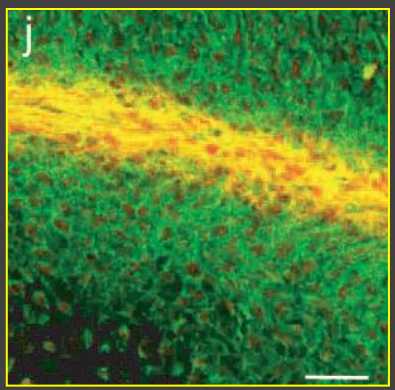
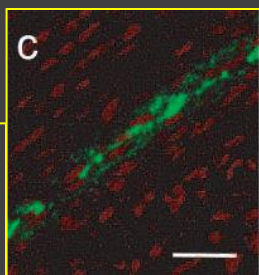
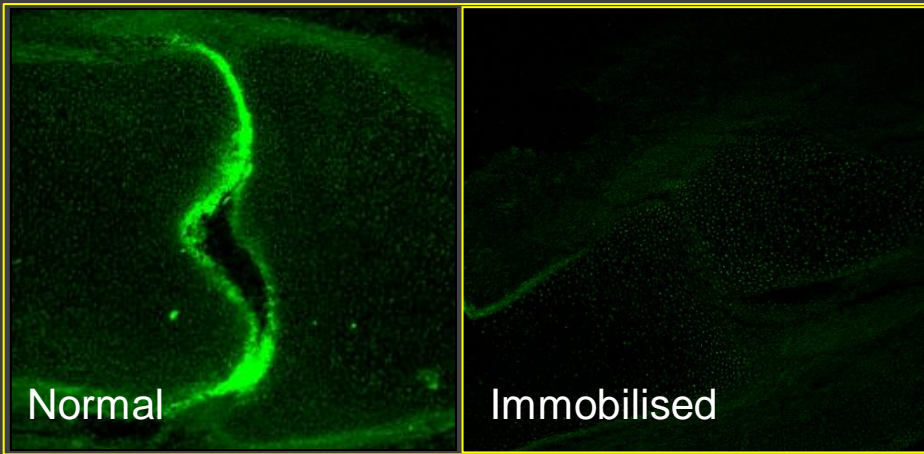
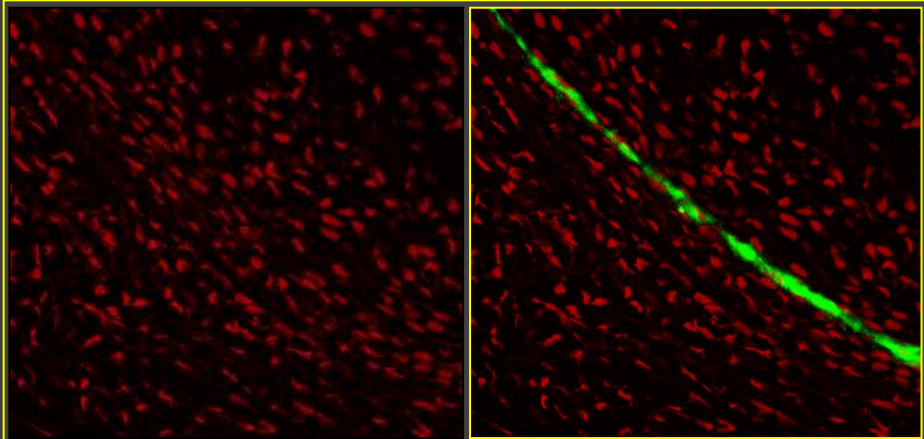


Stages 36-37



*Dowthwaite et al., Matrix Biol, 2003*  
*Bastow et al. JBC 2005*  
*Kavanagh et al. Dev Dyn. 2006*

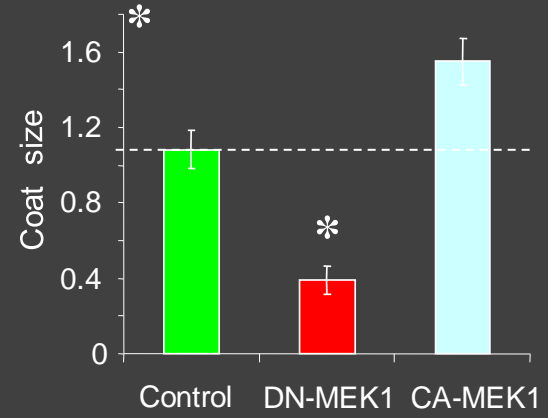
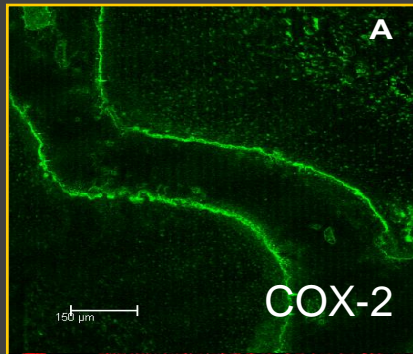
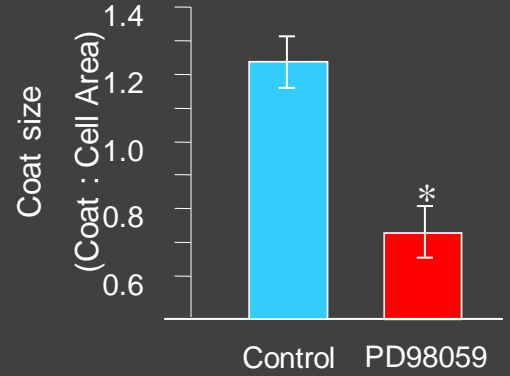
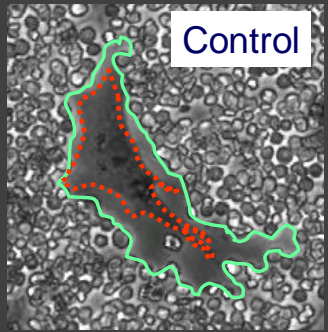
# Movement activates ERK selectively at forming joint surfaces



ERK active:  
cytoplasm

Co-loc<sup>n</sup>:  
polymerised actin

## ERK function in HA synthesis/binding



Phospho-ERK Propidium iodide

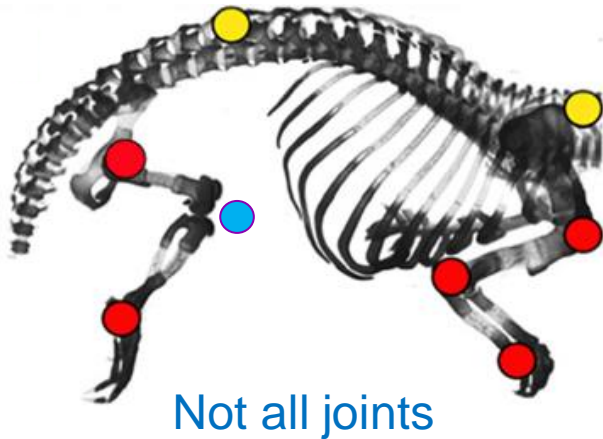
Bastow et al. JBC 2005

p38 kinase Lewthwaite et al., JBC. 2006

COX-2 Wheeler et el., under revreviw



# Muscle Contraction Is Necessary to Maintain Joint Progenitor Cell Fate

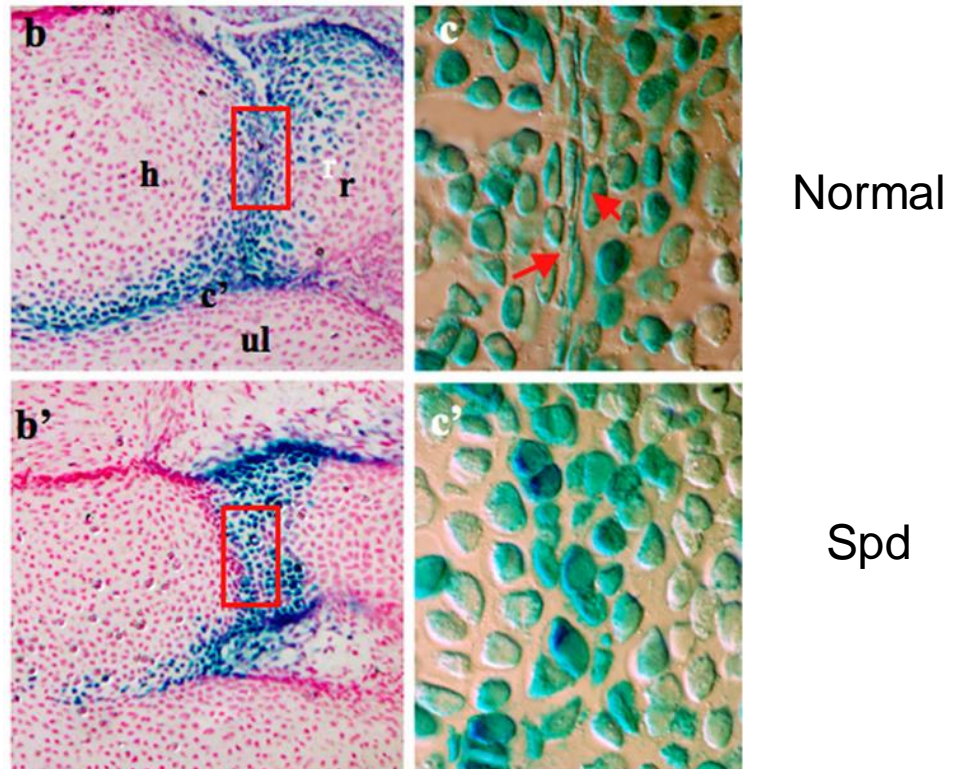


- splotch delayed mutant (Spd): Pax3 mutation defect in muscle progenitor cell migration
- Myf5/MyoD KO - no myoblast formation
- Spd: Reduced proliferation at forming joints

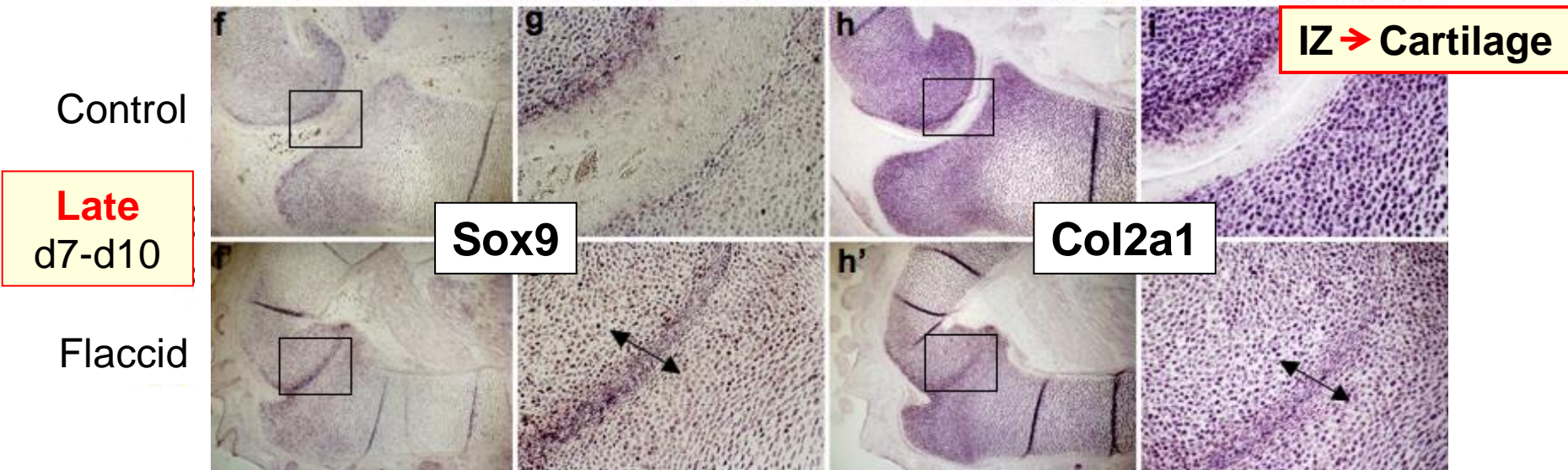
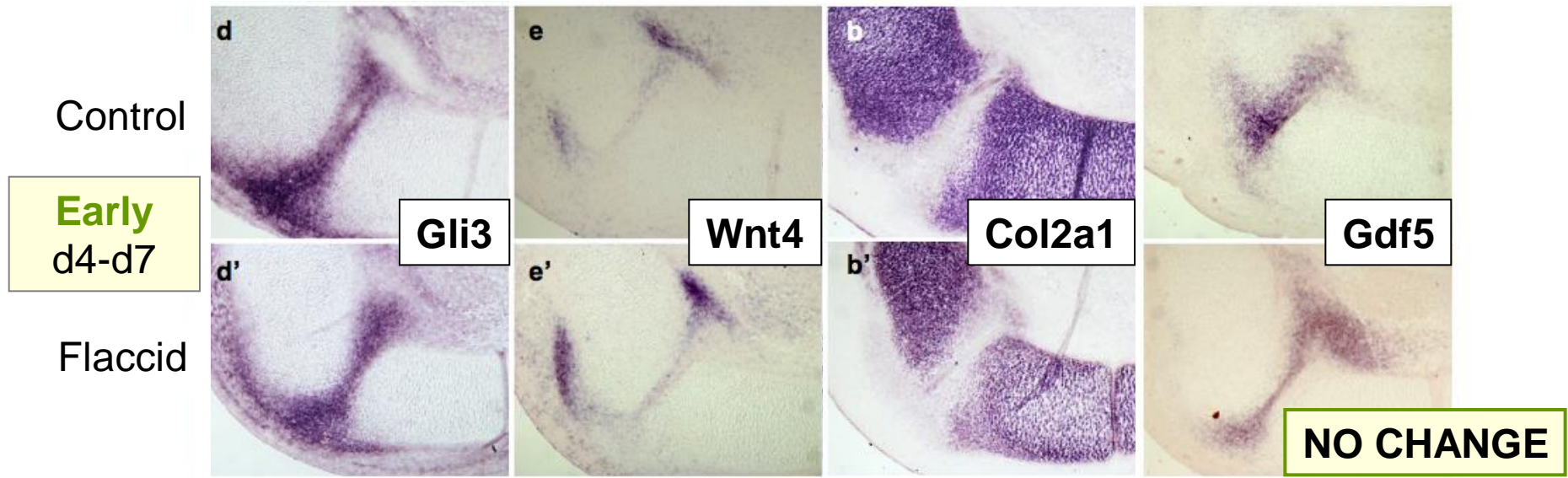
Gdf5-Cre mice crossed with R26R-lacZ reporter mice

- Joint progenitors lose normal behaviour & differentiate into chondrocytes

*Kahn et al., (2009)  
Dev Cell. 16:734-43*



*Mechanosensitivity of the interzone is acquired*

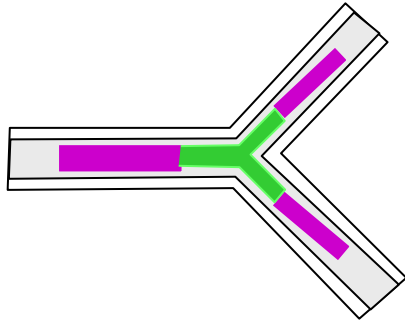


Joint formation but not patterning

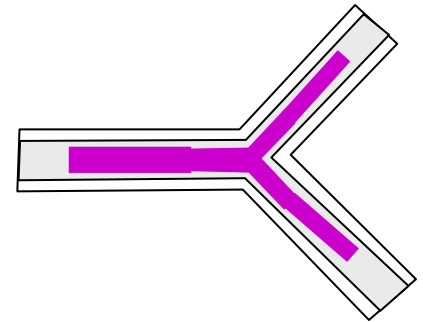
Yulia Shwartz & Elazar Zelzer (collaboration)



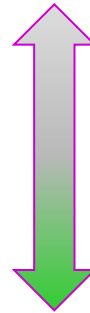
# Plasticity of interzone cells is key fate-determining step



Too many differentiate into chondrocytes (lost from interzone) then joints **fuse**

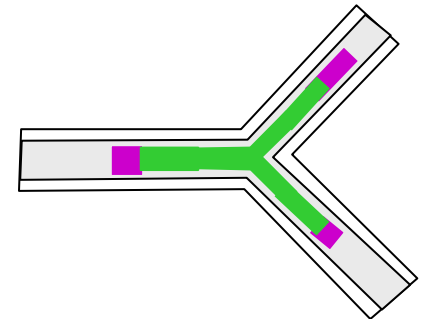


*Movement-induced mechanical stimulus*



*converge upon 'pro-inflammatory' signalling?*

Too many remain in interzone pool, then skeletal elements won't **grow** sufficiently

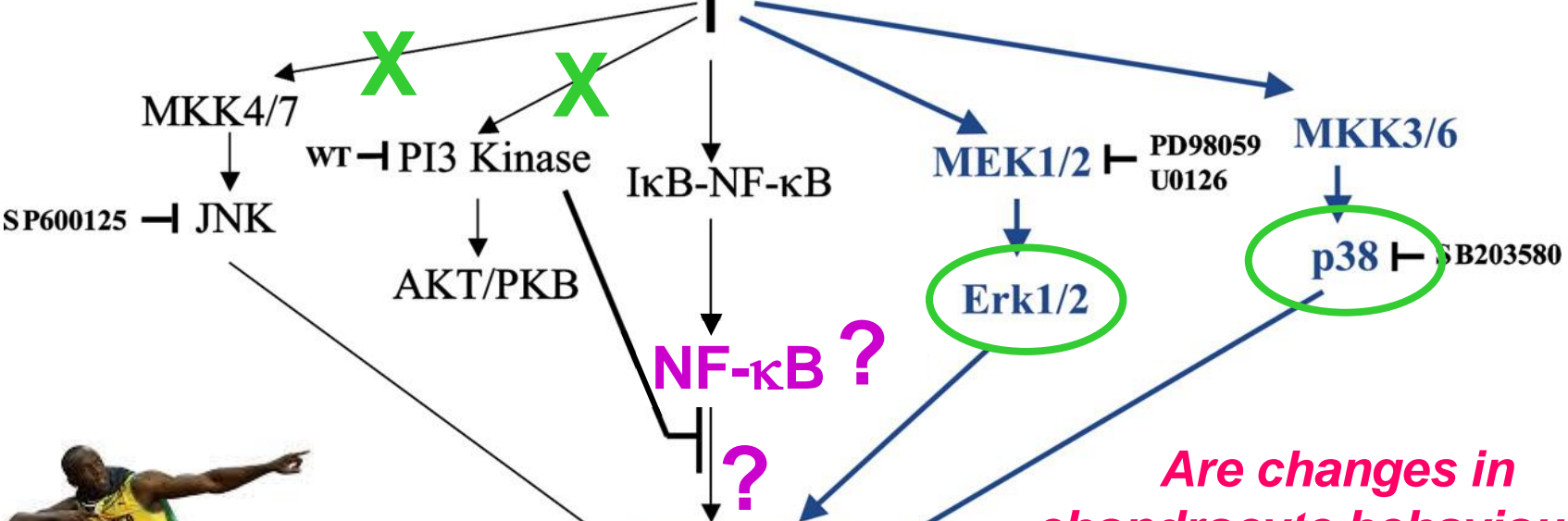


*Mechanisms responsive to movement that define these boundaries are crucial*

*Bastow et al., JBC, 2005 (ERK)  
Lewthwaite et al., JBC. 2006 (p38<sup>MAPK</sup>)  
Wheeler et al., submitted (COX-2)*

TNF- $\alpha$ , TGF- $\beta$ , IGF, IL1, IL8

Cell Membrane



*Are changes in chondrocyte behaviour in OA mechanically-guided?*

COX-2

NS398

PGE<sub>2</sub>

Inflammation **genomic instability**

**Plasticity!**



# NFkB signalling dominates gene expression in cartilage predisposition, onset and progression in OA-prone mice

*Poulet et al., 2012 A&R*

Str/ort	Str/ort	Str/ort
8wks	18wks	40wks
CBA	CBA	CBA
8wks	18wks	40wks

## OA predisposition – Str/ort v. CBA 8wks

139 up- and 0 down-regulated genes

Collagen VIII and XII  
Tenascin C  
MMP19  
Htra1  
TIMP1

IPA:

- Genetic
- Connective tissue disorders
- Cell death

Centred on **NFkB signalling**

Davidson et al, 2013 A&R  
Javaheri et al., 2015



Evgen

*Stable synthetic sulforaphane improves bone architecture & gait in Str/ort*

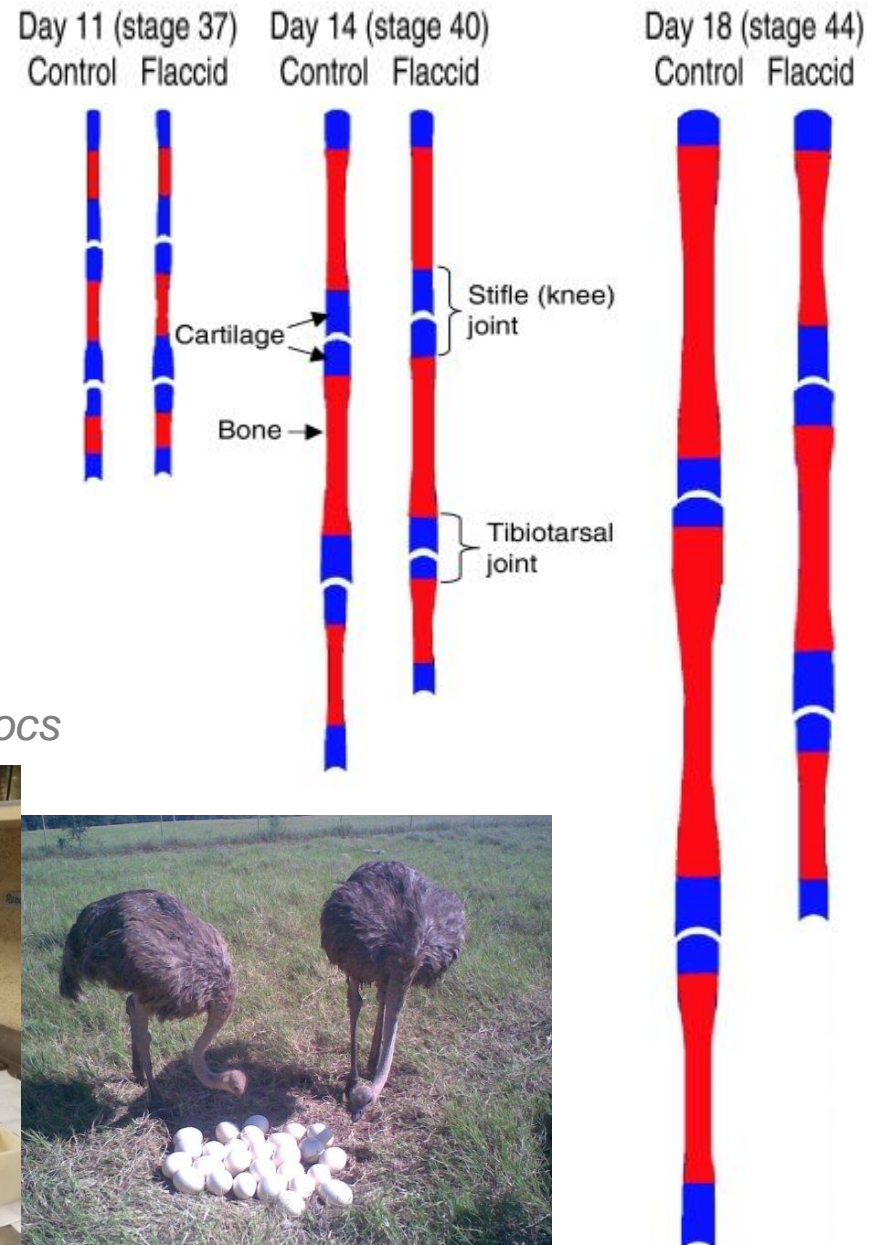


# Mechanical input required for joint cavitation & normal skeletal growth

- *Osborne et al., JMNI, 2002*
- *Lamb et al, IJEP, 2003*
- **Limb proportions?**

Andrea Pollard

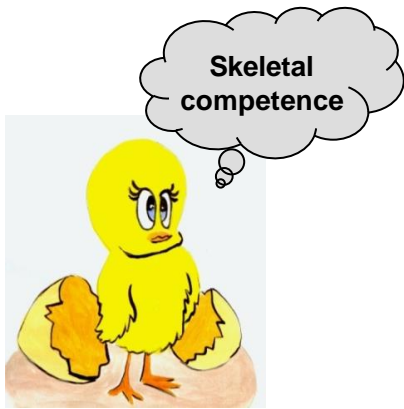
*Pollard et al, 2014 Journal of Anatomy*



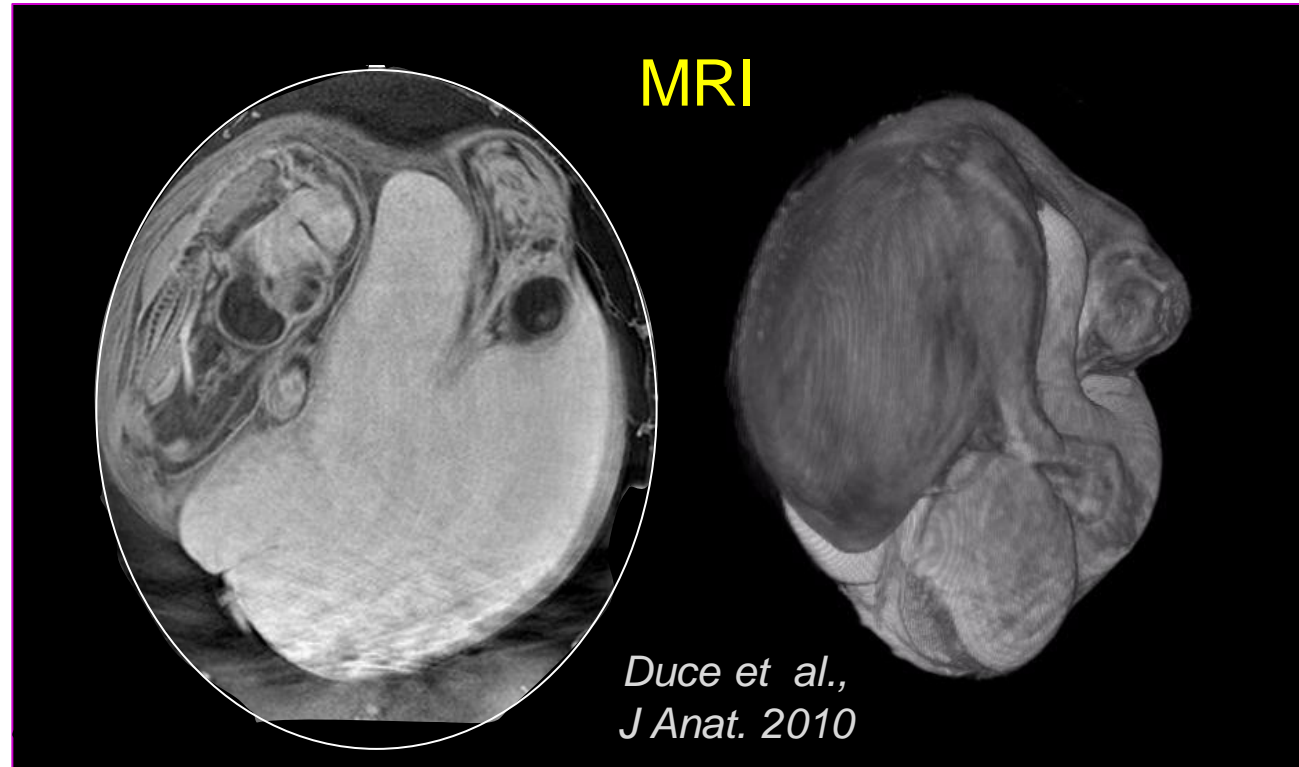
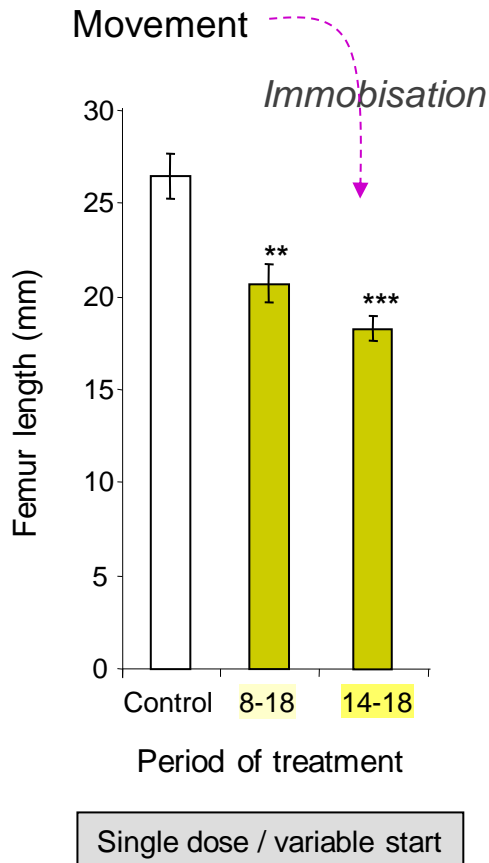
*Chick - Ostrich - Crocs*



*Gustavo Hernandez, Mexico*



- Embryo **movement starts early** in development
  - day 5 in chicken, week 7 in humans
  - evidence that this serves a **critical role**



- *Define critical time-points*
- *Determine if impact on skeletal growth is due to proximo-distal sequence of development*
- *Identify affected phases of endochondral ossification*



Unpublished data



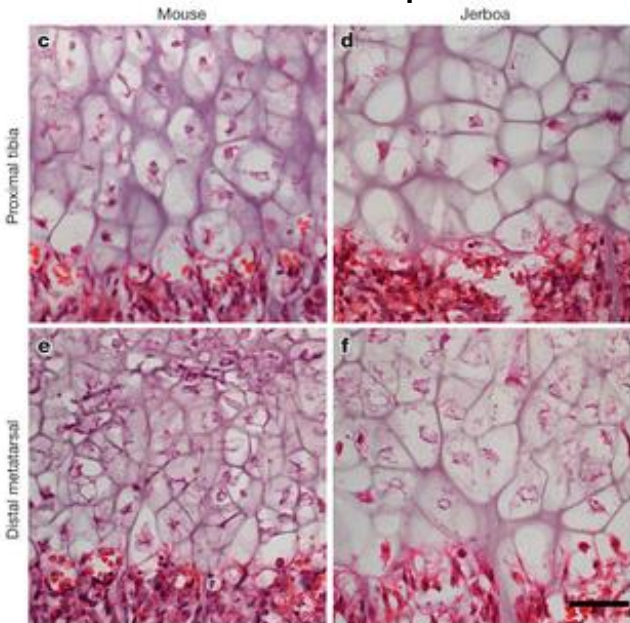
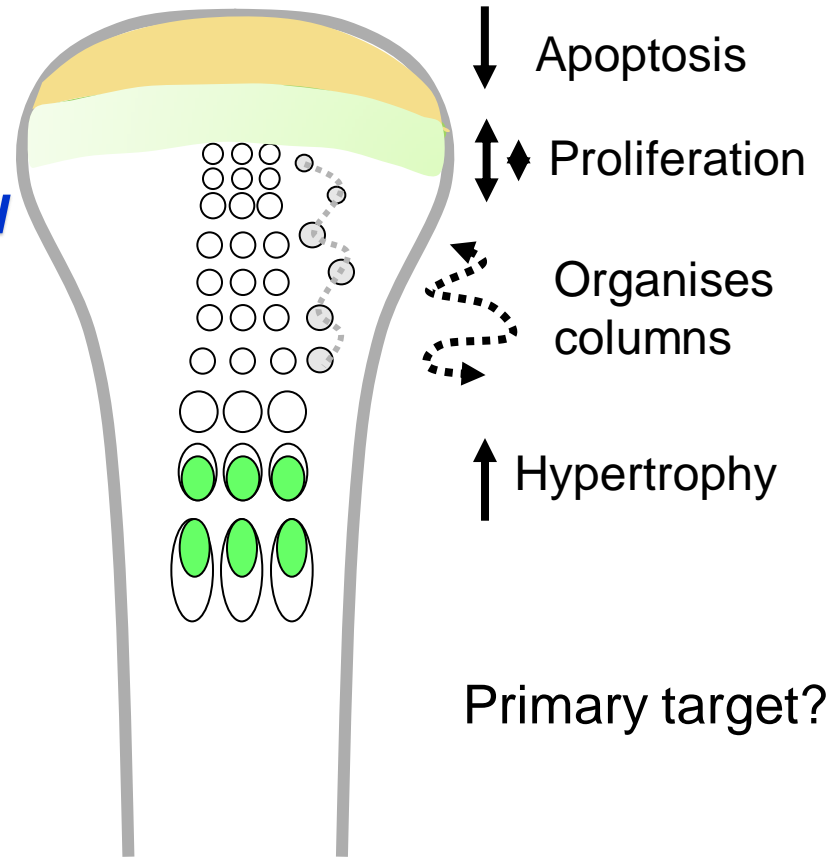
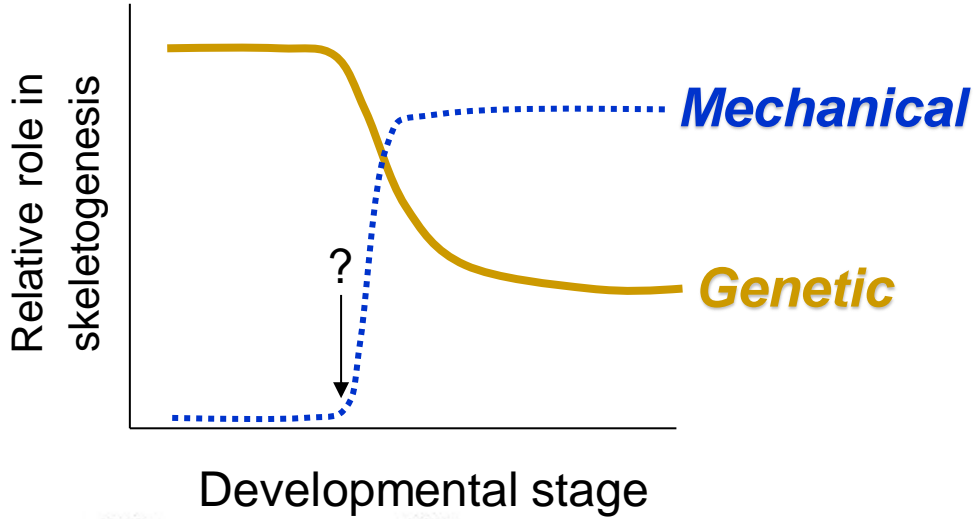


Unpublished data



Unpublished data

*Movement differentially drives skeletal element growth, after a certain developmental time-point, to modify limb proportionality*



PB?

Cooper et al.  
(2013) Nature



*Is the differential growth achieved in this final hypertrophy phase partly dependent on movement?*

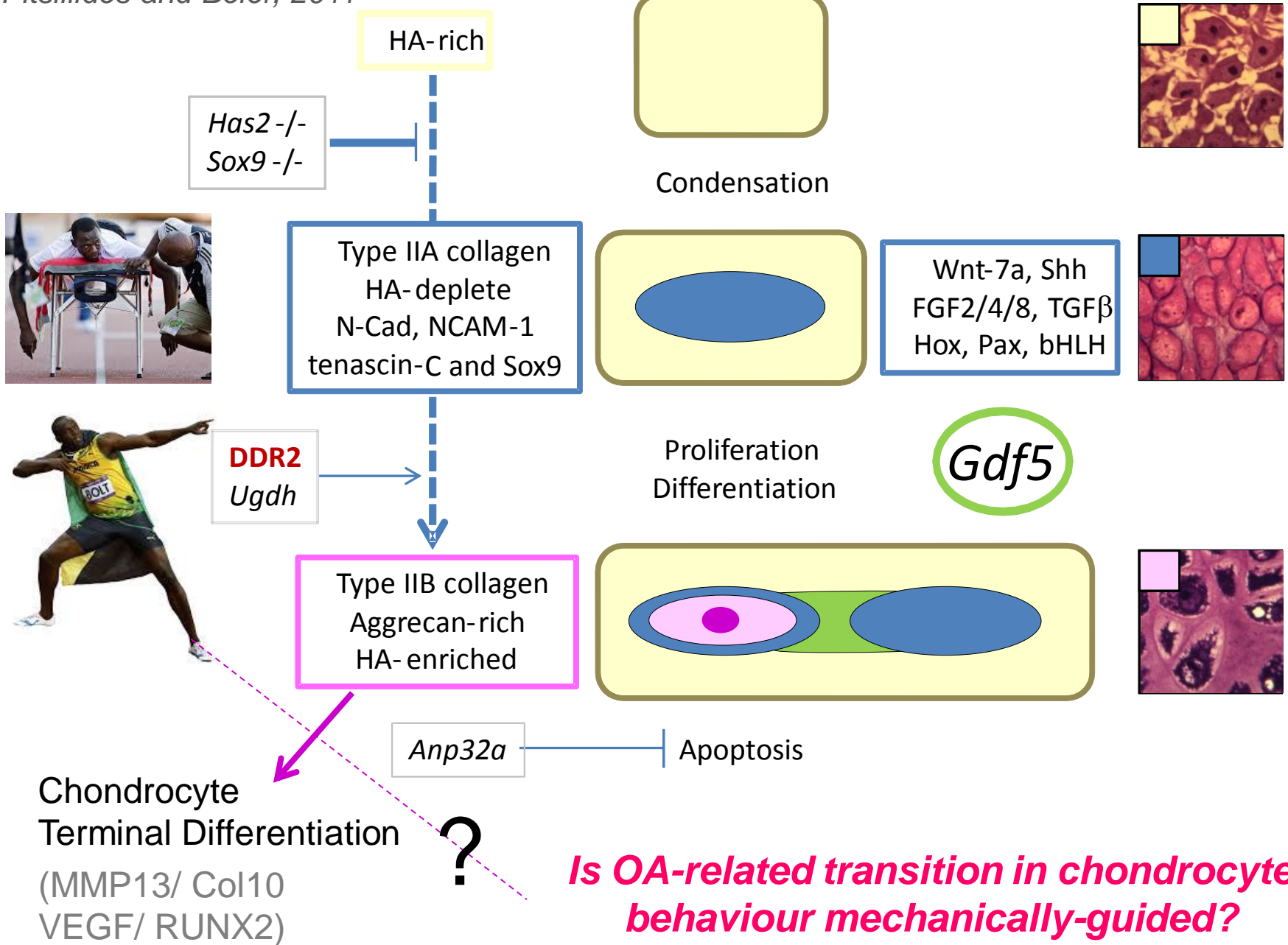




Unpublished data



Unpublished data

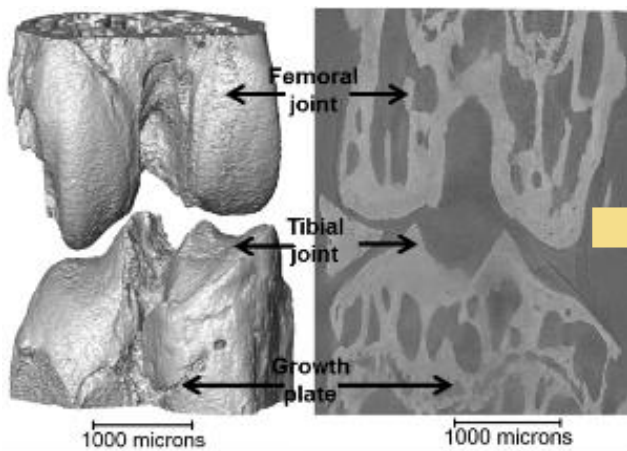


**Is OA-related transition in chondrocyte behaviour mechanically-guided?**

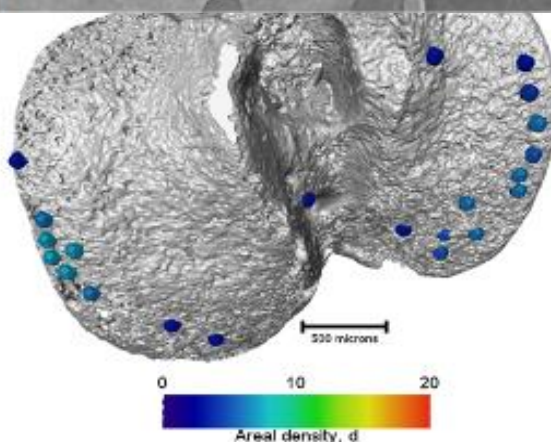
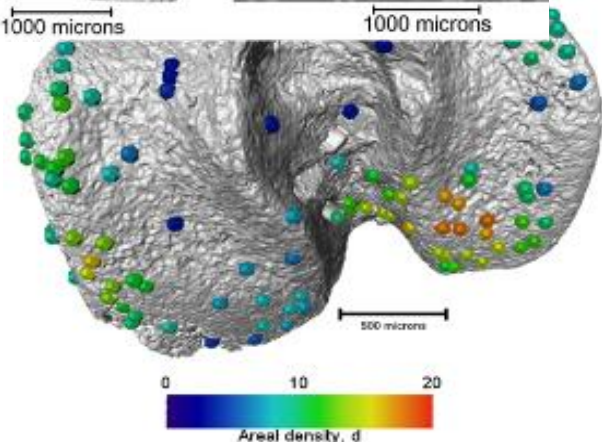
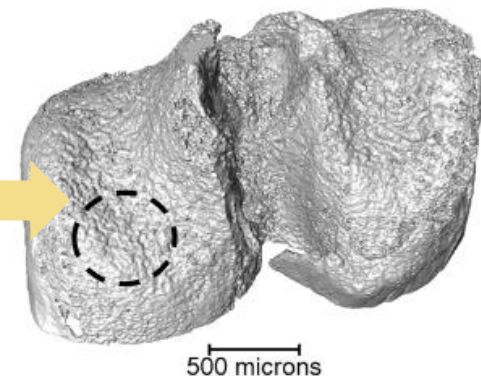
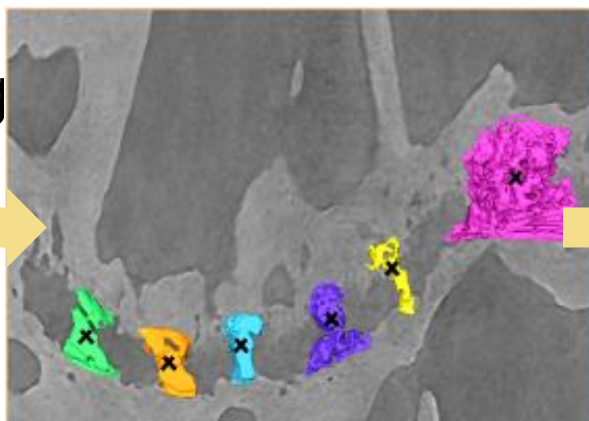




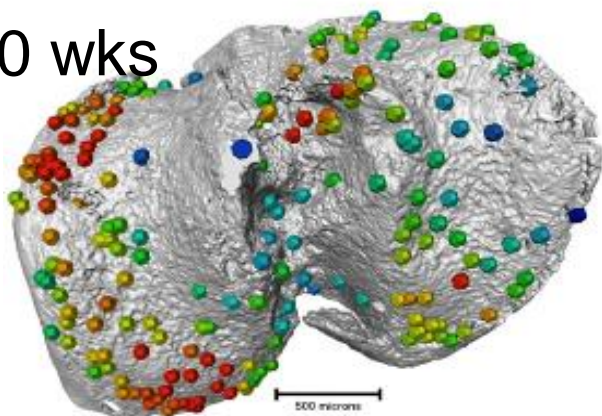
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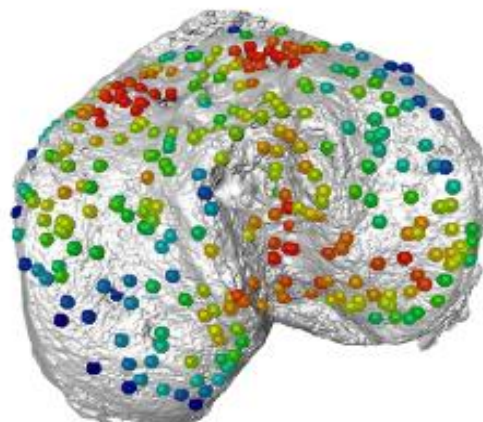
Bridge  
in  
CBA



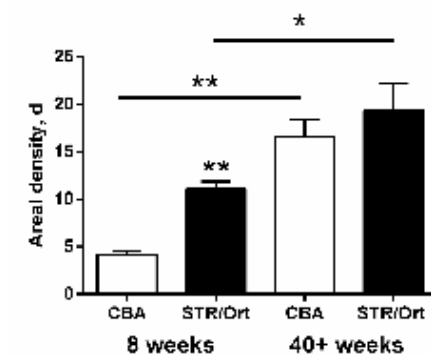
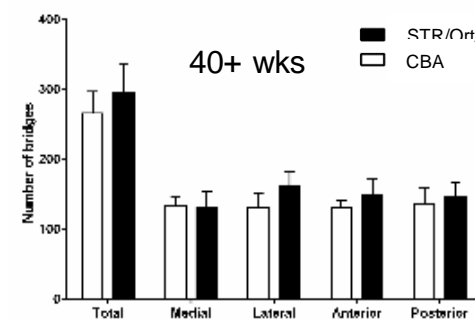
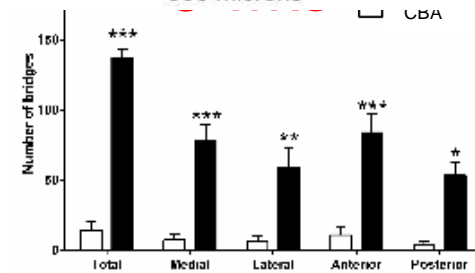
40 wks



STR/Ort



CBA



Katherine Staines  
Andrea Pollard  
Mark Hopkinson  
Gustavo Hernandez  
Blandine Poulet

Gary Dowthwaite  
Anne Osborne  
Emma Kavanagh  
Katherine Lamb  
Edward Bastow  
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