KU LEUVEN

Christa Maes

Lab of Skeletal Cell Biology and Physiology (SCEBP)
Skeletal Biology and Engineering Research Center (SBE)
Department of Development and Regeneration, KU Leuven

ECTS
European Calcified Tissue Society

CONFLICT OF INTEREST

Christa Maes

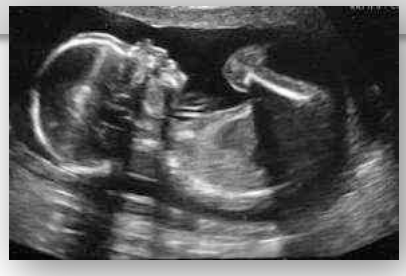
I declare that I have no potential conflict of interest.

What is New? The Year in Review:

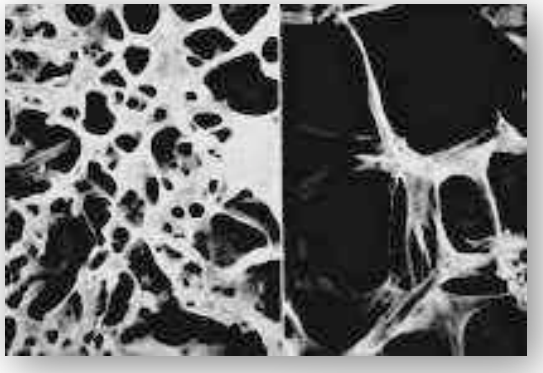
Bone Basic Science Highlights

Basic & Translational research models (mouse)

Development
Bone growth

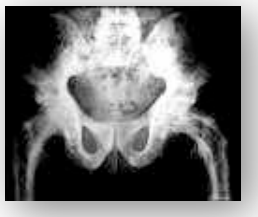


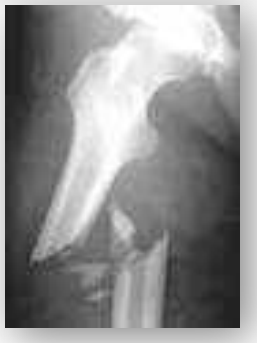
Bone formation & homeostasis



Fracture repair & TE

Metastasis

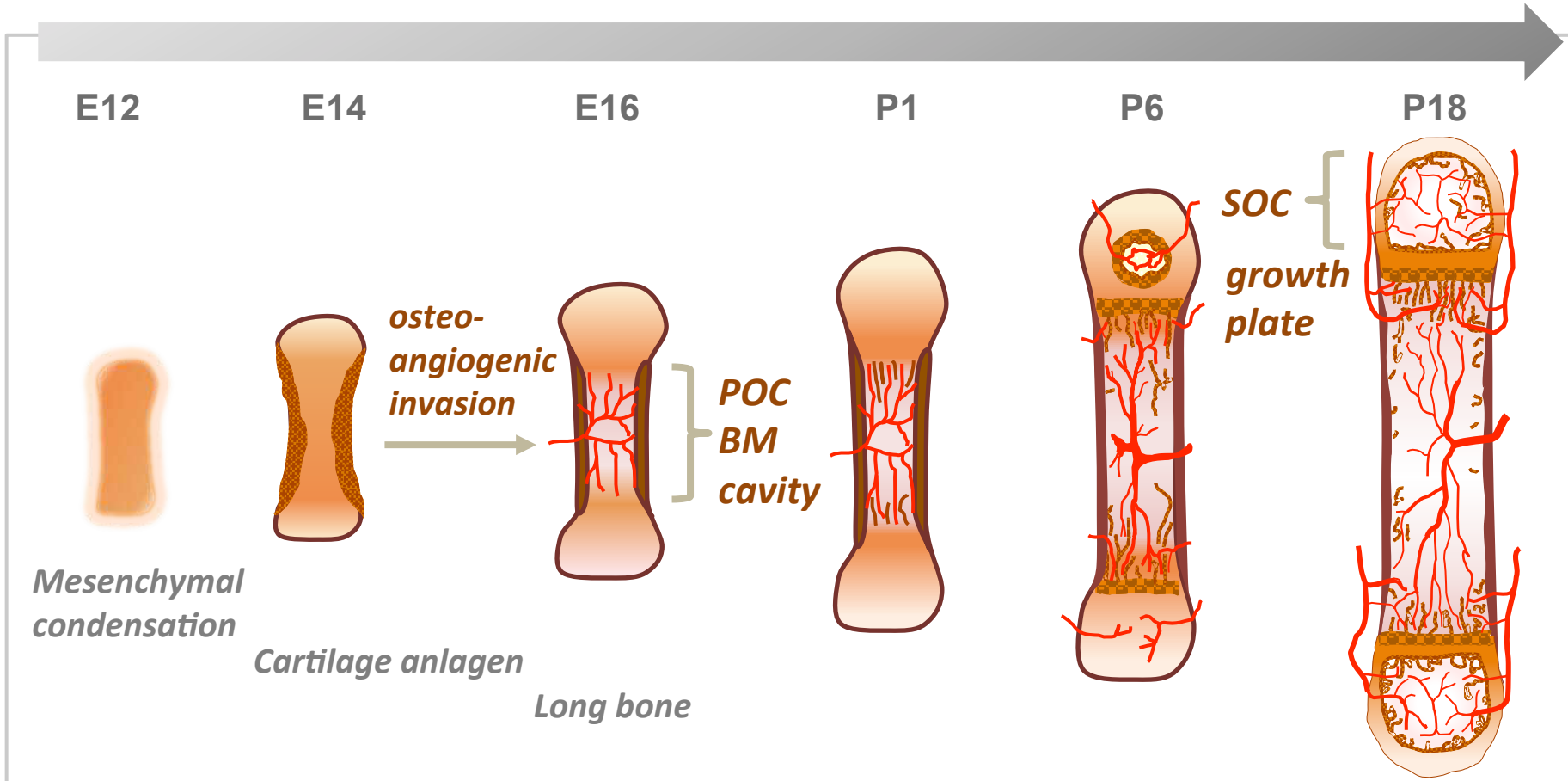




- **Limited** selection of papers (that can be discussed in the time frame of 30 mins)
- Published in 2015-2016
- Research papers covering diverse aspects of skeletal cell biology and physiology (…*with a personal bias*…)
- Too much interesting material for 30 mins!
- **Apologies to all authors whose wonderful work is not presented here!**

Long bone development by endochondral ossification

Chondrogenesis	*Primary ossification*	*Longitudinal bone growth*	*Secondary ossification*



FGF18-mediated autophagy regulates bone growth in mice (Cinque et al., Nature, Dec 2015)

LETTER

Cinque et al., Nature 528:272-275, Dec 2015

doi:10.1038/nature16063

FGF signalling regulates bone growth through autophagy

Laura Cinque[1,2*], Alison Forrester[1,2,3*], Rosa Bartolomeo[1,2], Maria Svelto[1,2,3], Rossella Venditti[1], Sandro Montefusco[1], Elena Polishchuk[1], Edoardo Nusco[1], Antonio Rossi[4], Diego L. Medina[1], Roman Polishchuk[1], Maria Antonietta De Matteis[1] & Carmine Settembre[1,2,3]

- **Autophagy** is the conserved process whereby **aggregated proteins, intracellular pathogens, and damaged organelles are degraded and recycled**
- **Autophagic structures** can also act as **hubs for the spatial organization of recycling and synthetic processes in secretory cells**

L.J. Hocking, C. Whitehouse, and M.H. Helfrich
"Autophagy: A New Player in Skeletal Maintenance?" JBMR 2012

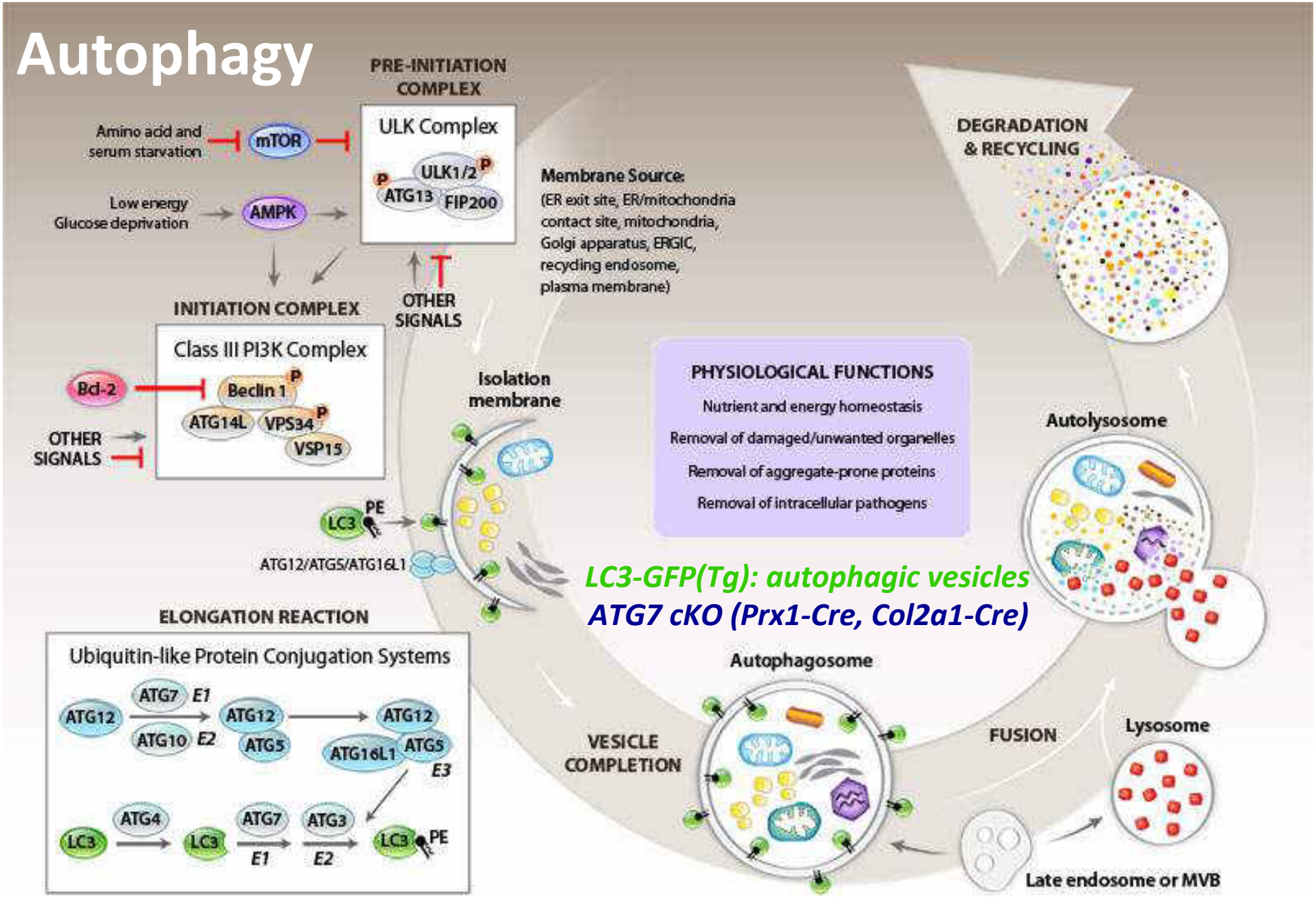
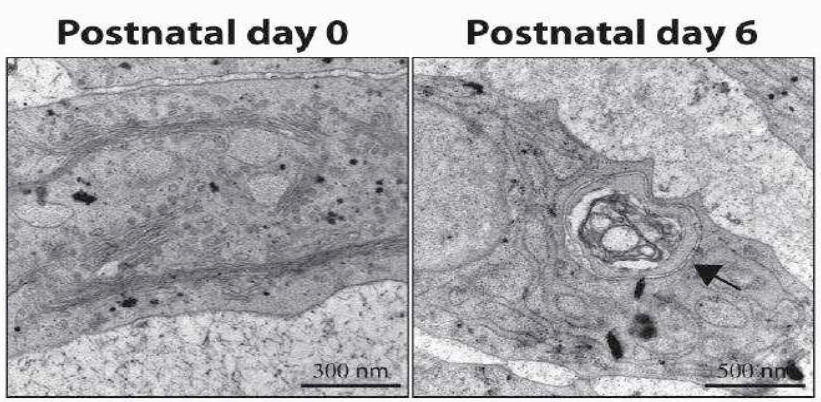
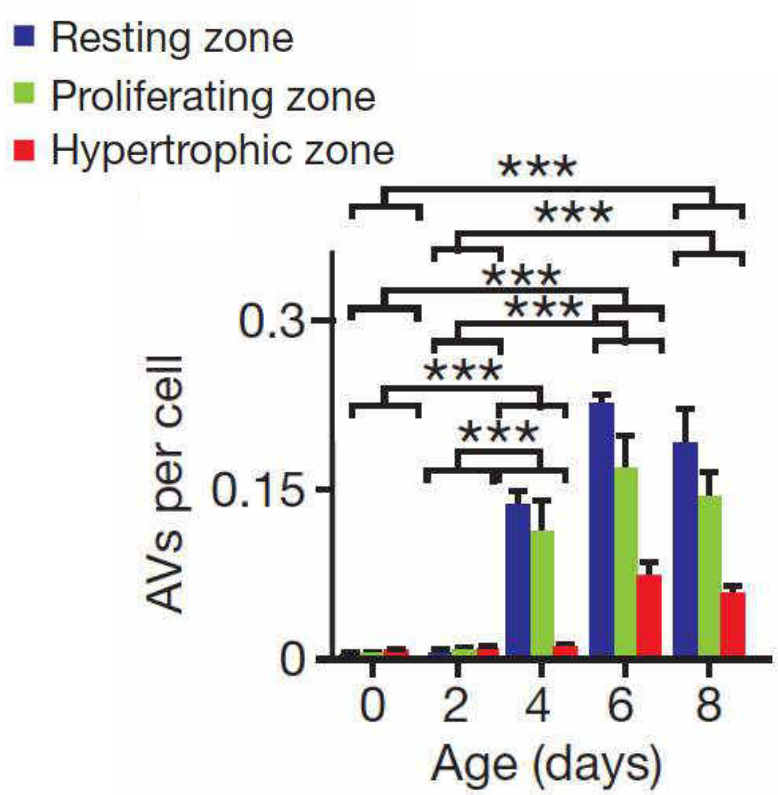


Image: Center for Autophagy Research, Levine Laboratory, UT Southwestern Medical Center, Dallas

LC3-GFP(Tg): autophagic vesicles

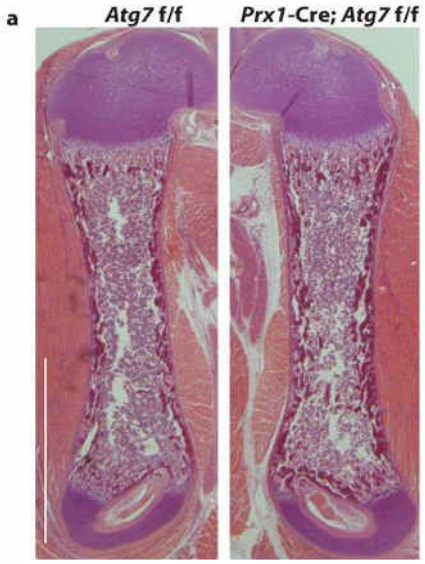




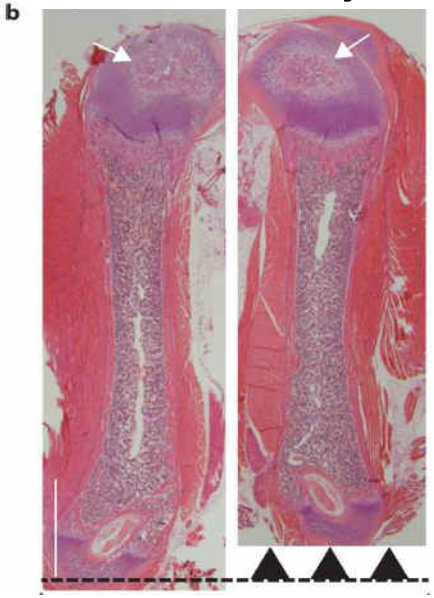
Cinque et al., Nature 528:272-275, Dec 2015

ATG7 cKO (Prx1-Cre, Col2a1-Cre)

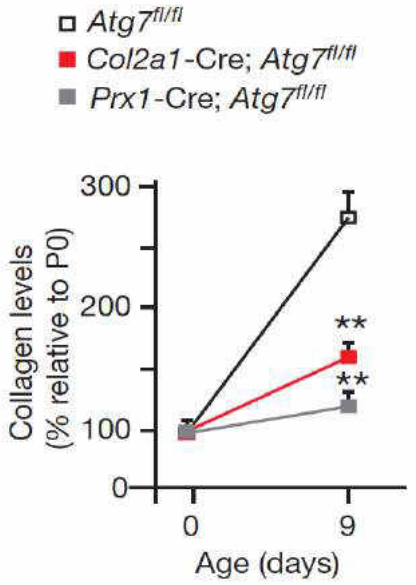
Postnatal day 6



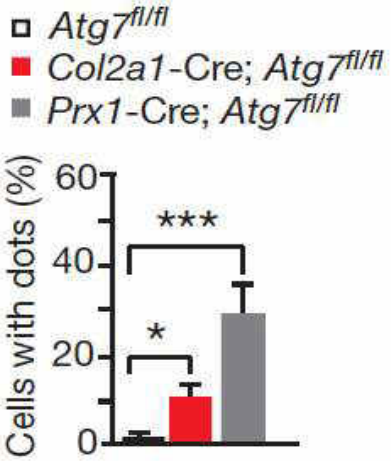
Postnatal day 9



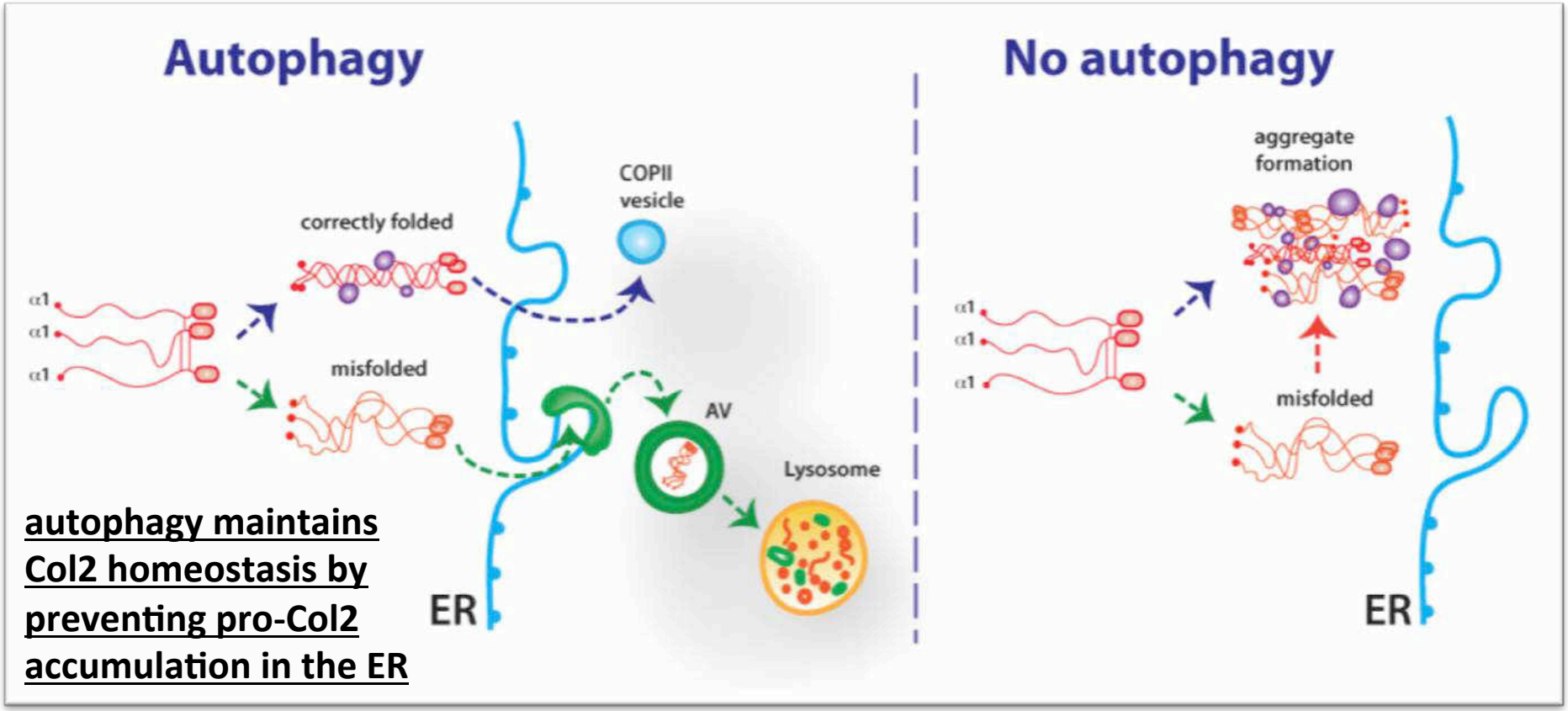
↓ Col2 levels in cartilage



↑ Intracellular pro-Col2 accumulation



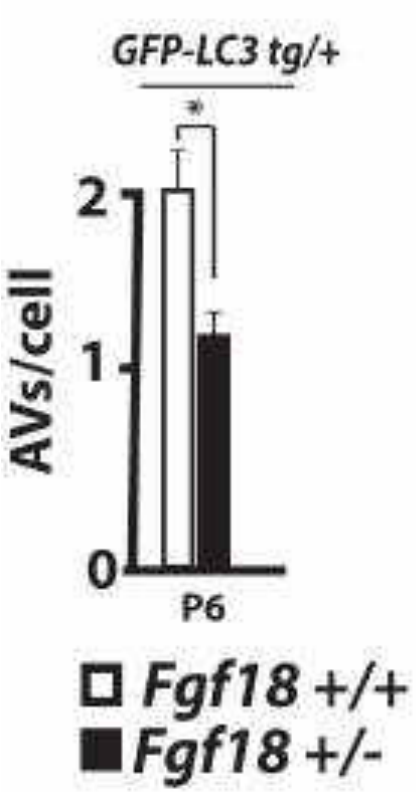
Autophagy regulates postnatal bone growth in part by controlling the secretion of type II collagen in chondrocytes

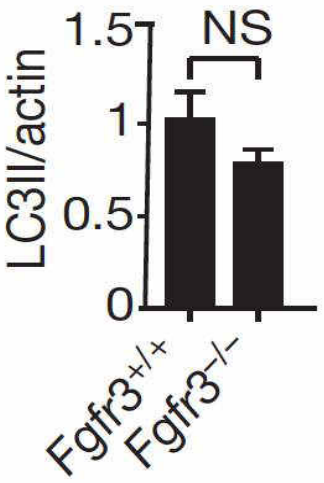


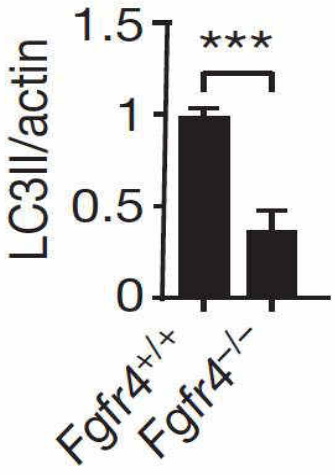
autophagy maintains Col2 homeostasis by preventing pro-Col2 accumulation in the ER

Autophagy in chondrocytes is regulated by FGF-18/FGFR4 signaling

LC3-GFP(Tg): autophagic vesicles

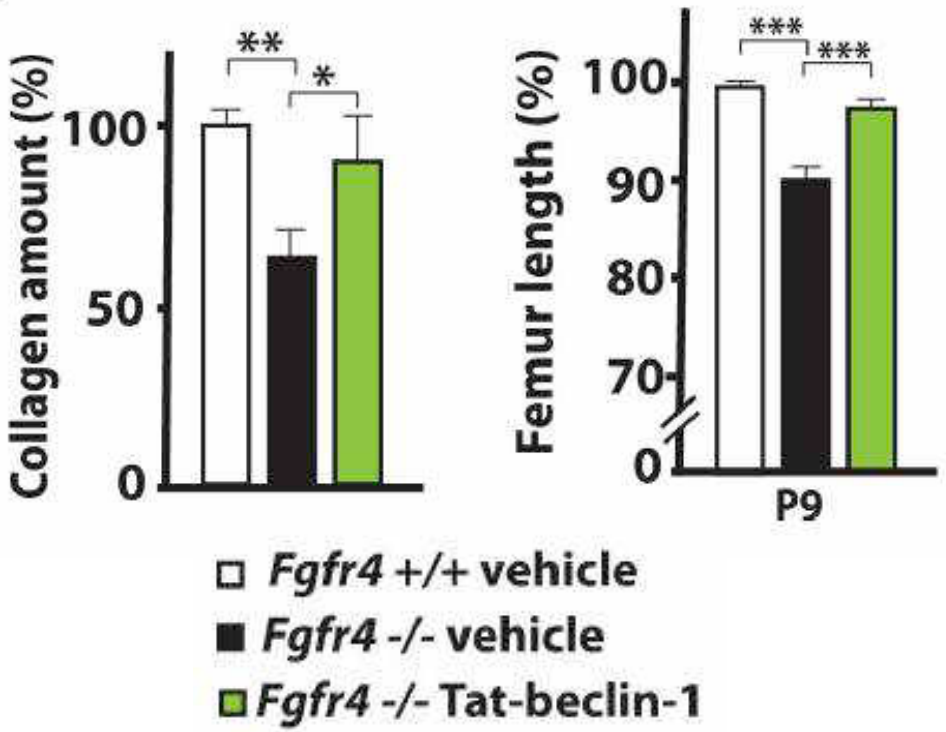




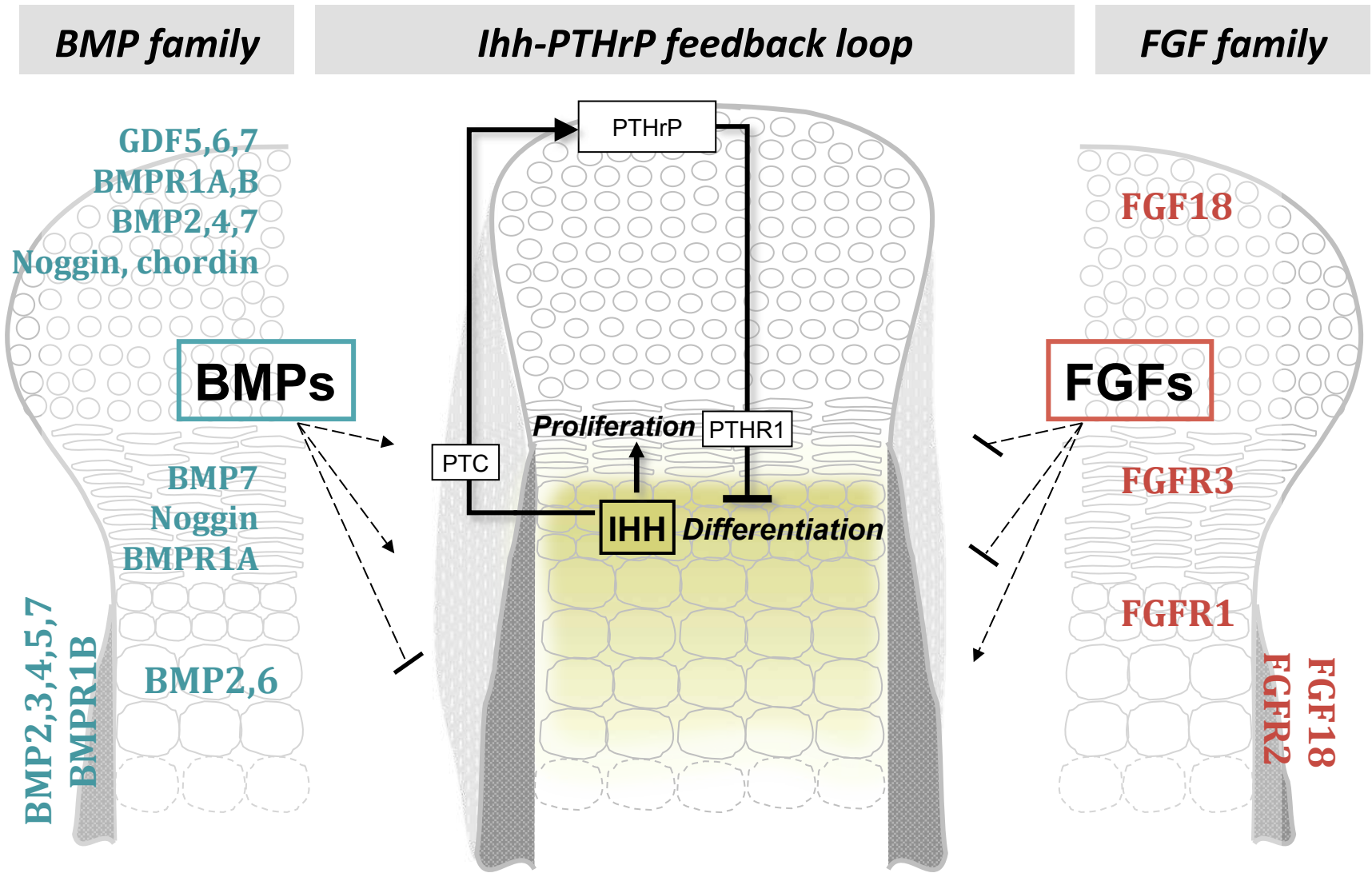


+Tat-beclin-1: autophagy-inducing peptide

➔ ***Rescue of collagen secretion defect***



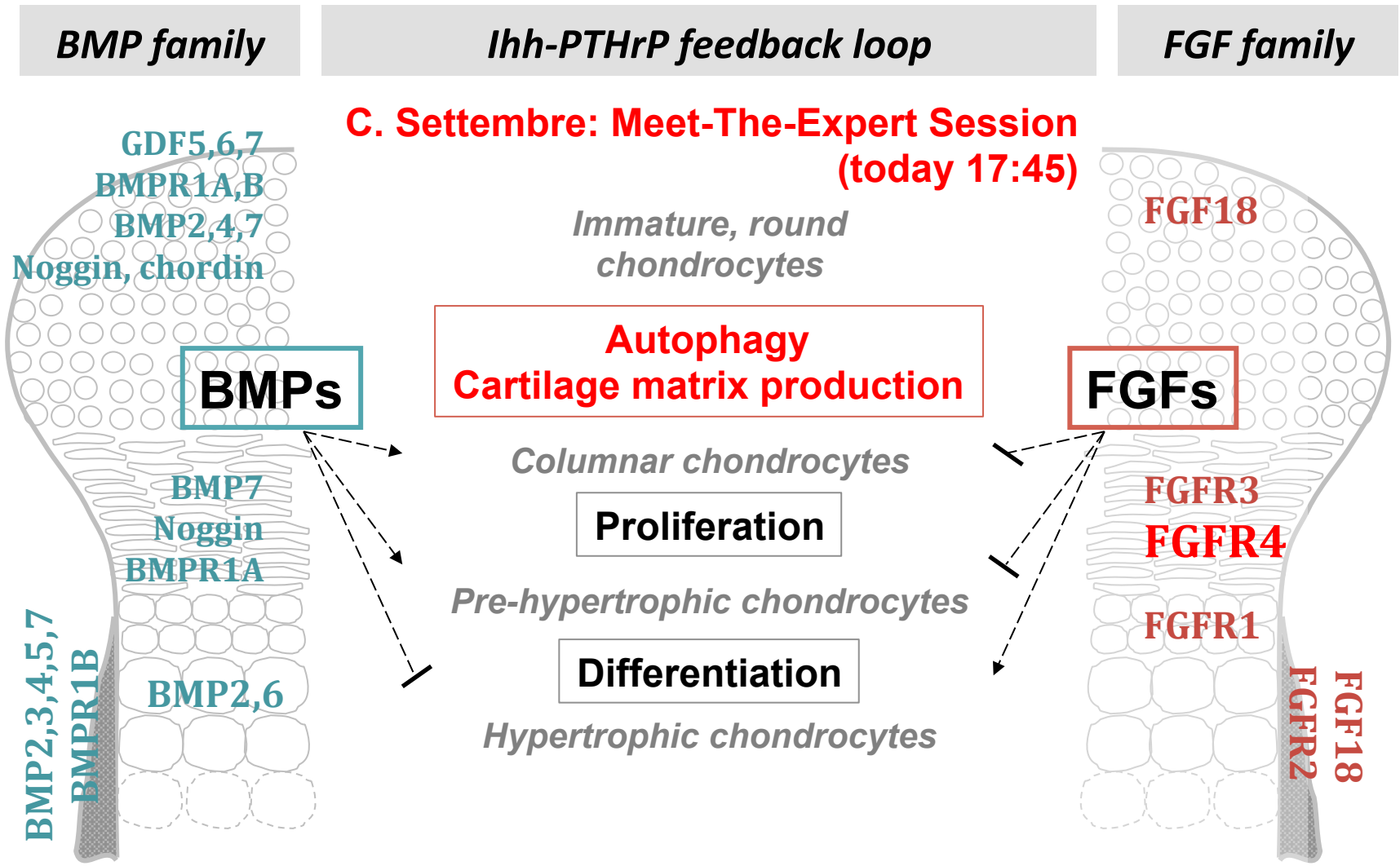
Interacting growth factor pathways control proliferation and differentiation of growth plate chondrocytes



Maes C., Sem Cell Dev Biol 2016 (in press)

"Signaling Pathways Effecting Crosstalk Between Cartilage and Adjacent Tissues"

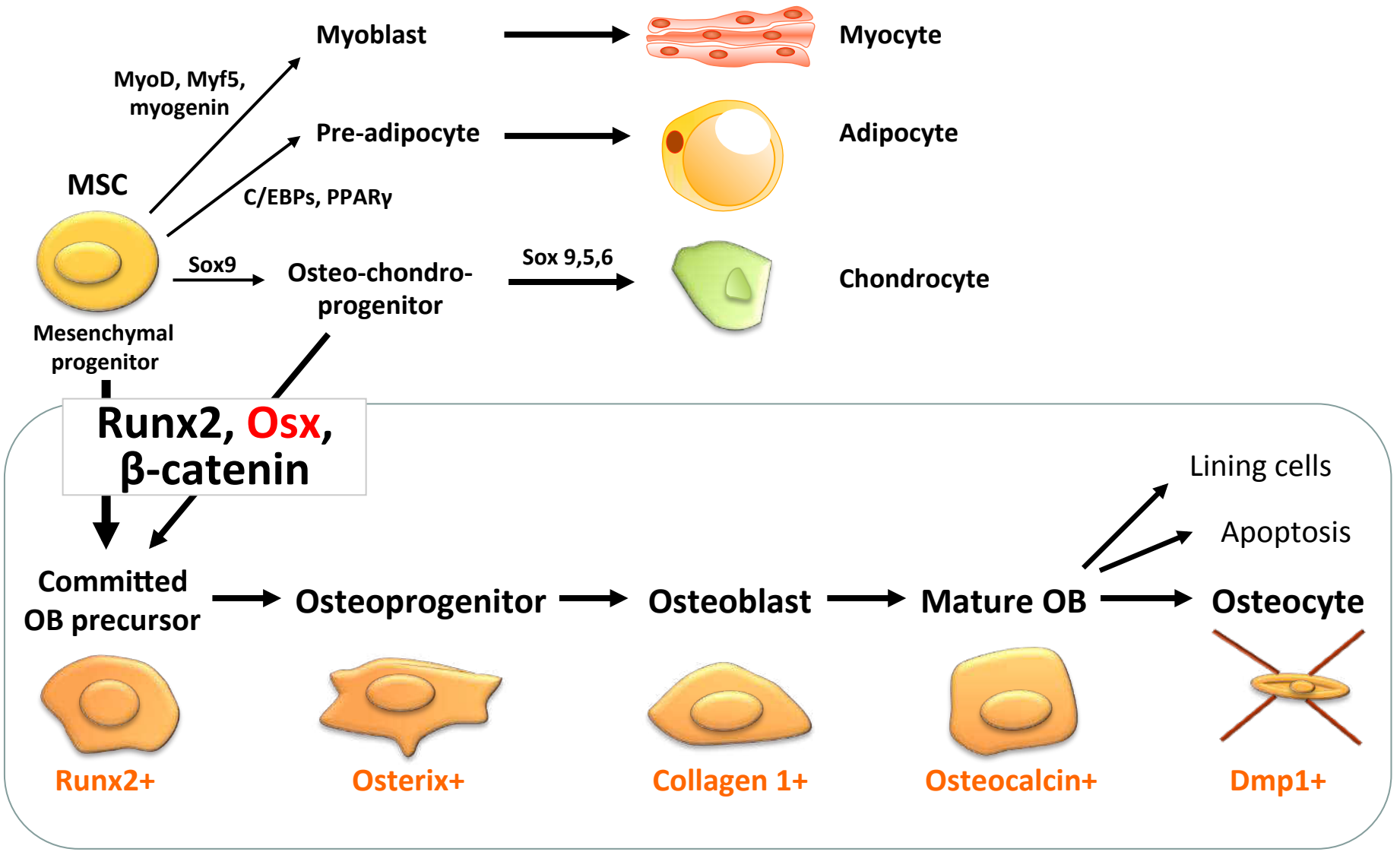
Interacting growth factor pathways control proliferation and differentiation of growth plate chondrocytes



Maes C., Sem Cell Dev Biol 2016 (in press)

"Signaling Pathways Effecting Crosstalk Between Cartilage and Adjacent Tissues"

Osteoblast differentiation

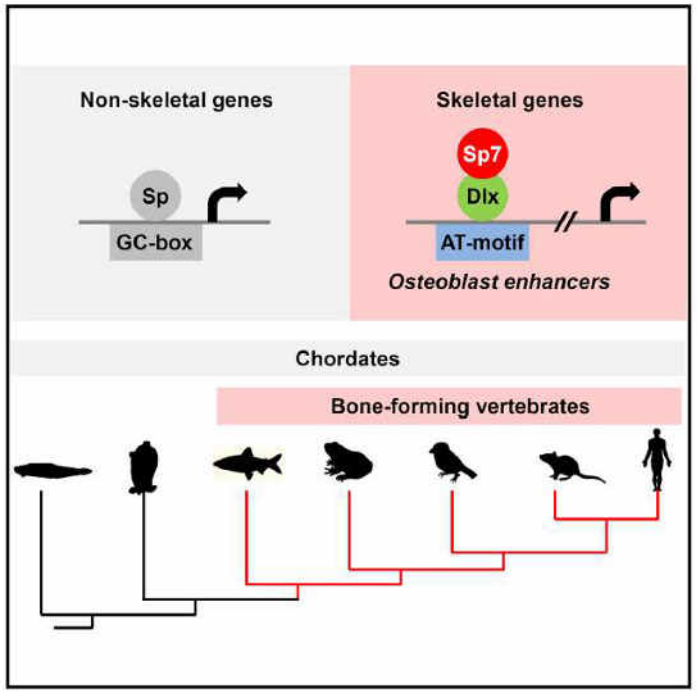


Article

Developmental Cell

Sp7/Osterix Is Restricted to Bone-Forming Vertebrates where It Acts as a Dlx Co-factor in Osteoblast Specification

Graphical Abstract



Authors

Hironori Hojo, Shinsuke Ohba, Xinjun He, Lick Pui Lai, Andrew P. McMahon

In Brief

Unlike other Sp family members, which directly bind a consensus GC box, Hojo et al. show that Sp7/Osterix, a master transcriptional regulator specifying bone-forming osteoblasts, engages targets indirectly, through Dlx factors bound at AT-rich motifs. Cross-species analysis suggests that an Sp7-like variant arose in a common ancestor of bone-forming vertebrates.

Sp7/Osterix:

Crucial osteoblastogenic TF

… but…

The osteogenic regulatory program determinants, target genes and mechanisms of action are poorly understood

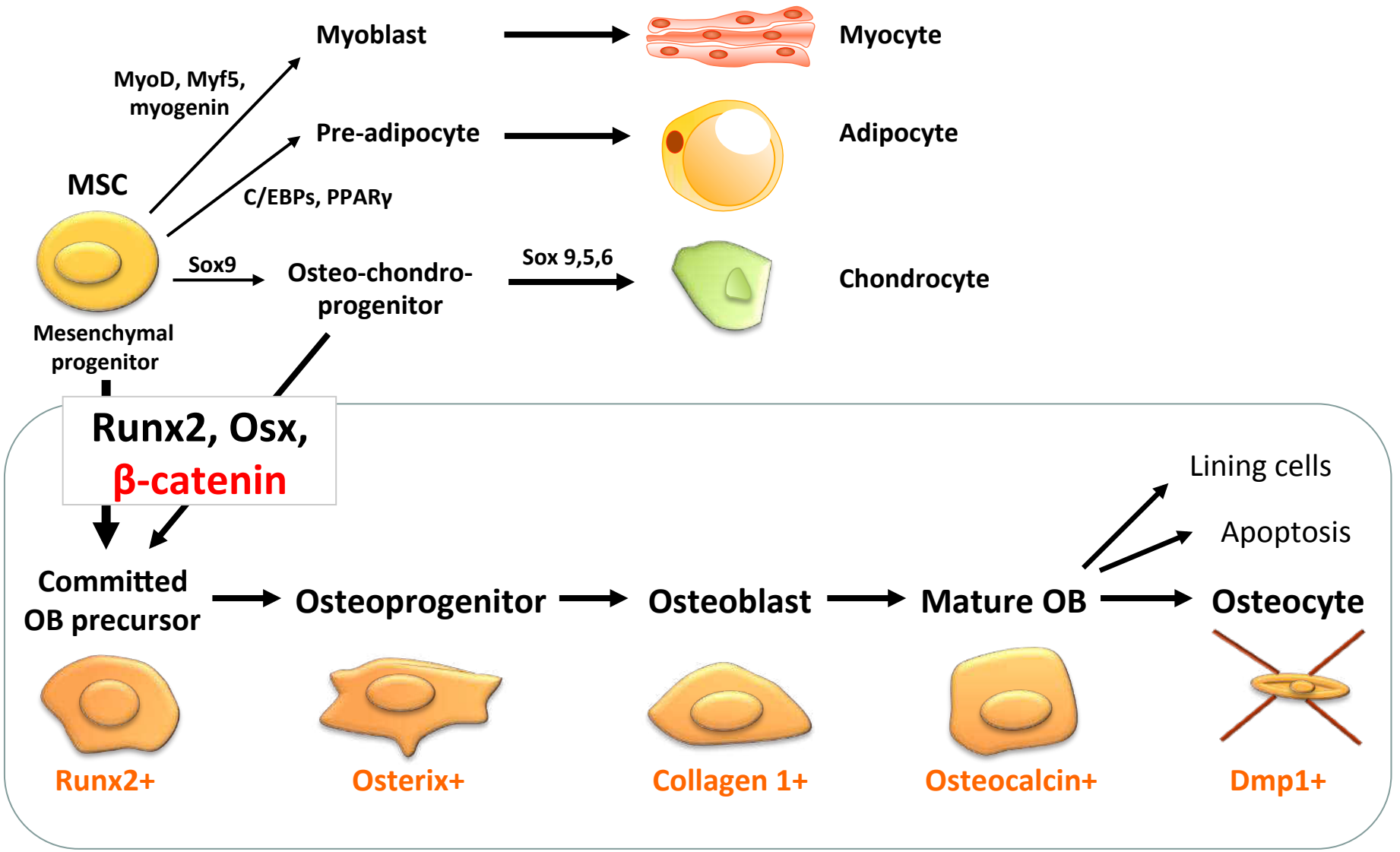
Highlights

- Sp7 genome-wide analysis identified osteoblast enhancers in calvarial osteoblasts
- Motif recovery and functional analysis indicate Sp7 acts through an AT-rich motif
- Sp7 indirectly engages the AT-rich motif through a Dlx complex
- The Sp7 Sp family variant correlates with the emergence of bone-forming vertebrates

***Sp7-Biotin-3xFLAG* knock-in mouse**
➔ Sp7 chromatin immuno-precipitation sequencing analysis (primary calvaria OBs P1 mice)

Hojo et al., Dev Cell, May 2016

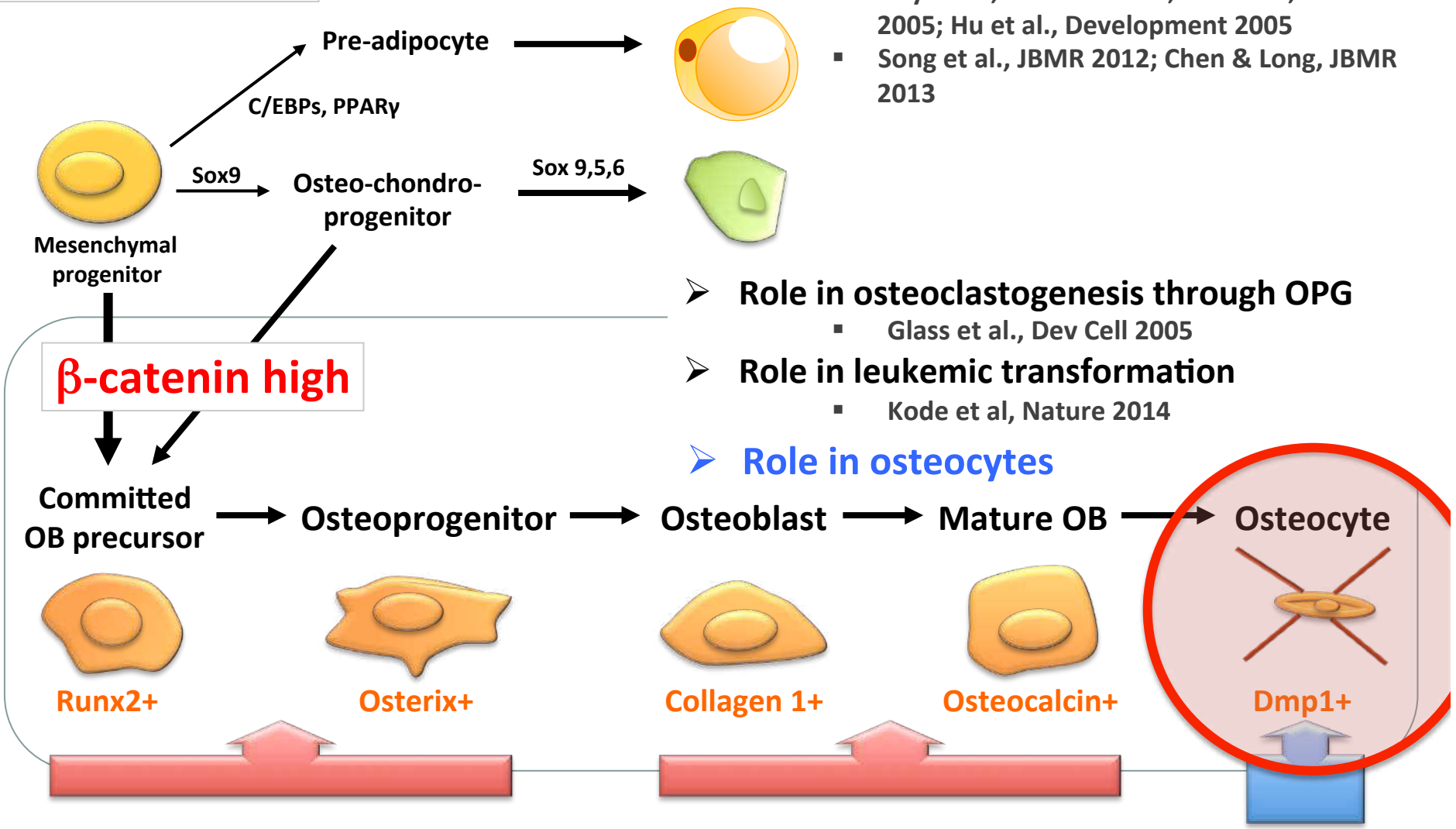
Osteoblast differentiation



WNT/β-catenin signaling in osteoblast lineage cells

- L-O-F / G-O-F β-catenin in mice

β-catenin low

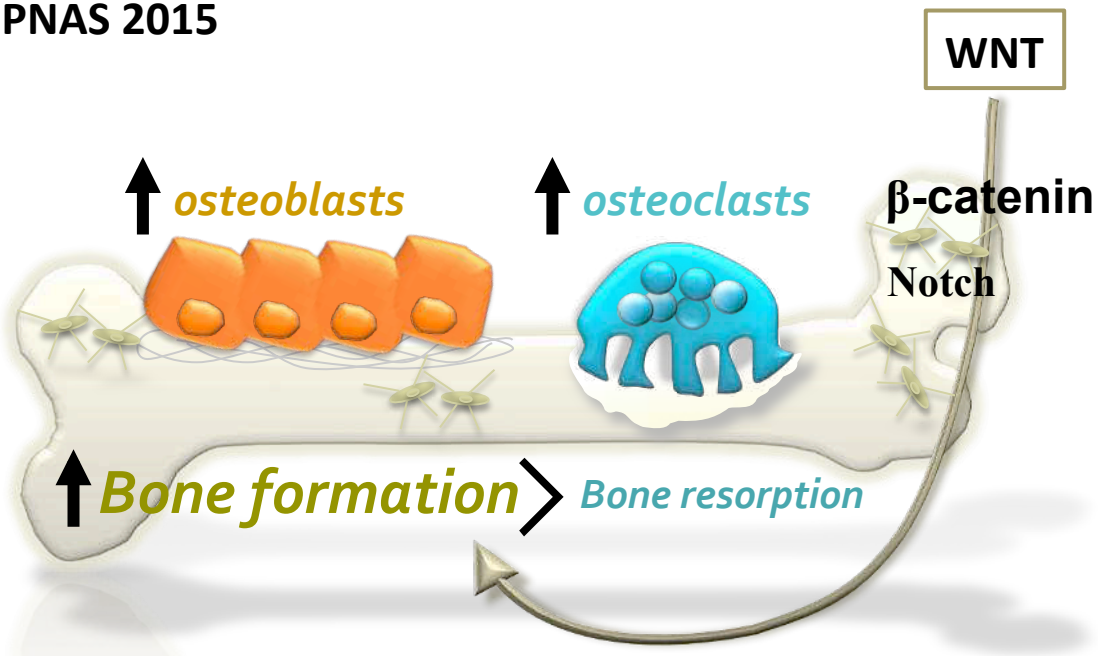


- Crucial role for β-catenin in the cell fate decision of mesenchymal progenitors
  - Day et al., Dev Cell 2005; Hill et al., Dev Cell 2005; Hu et al., Development 2005
  - Song et al., JBMR 2012; Chen & Long, JBMR 2013
- Role in osteoclastogenesis through OPG
  - Glass et al., Dev Cell 2005
- Role in leukemic transformation
  - Kode et al, Nature 2014
- Role in osteocytes

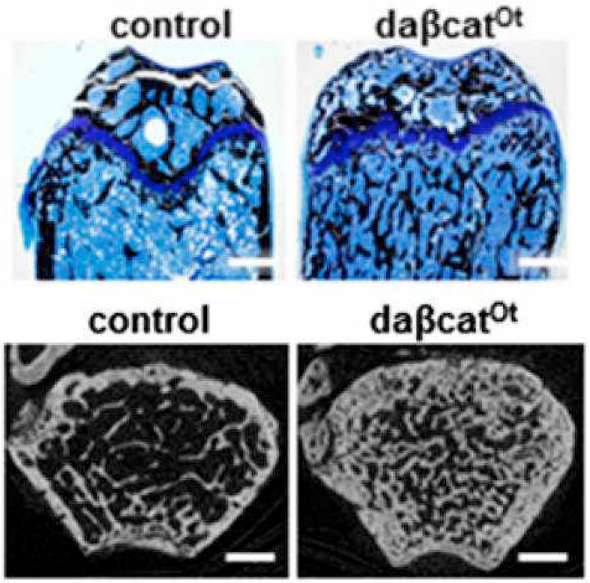
"Osteocytes mediate the anabolic actions of canonical Wnt/β-catenin signaling in bone"

Xiaolin Tu, Jesus Delgado-Calle, Keith W. Condon, Marta Maycas, Huajia Zhang, Nadia Carlesso, Makoto M. Taketo, David B. Burr, Lilian I. Plotkin, and Teresita Bellido

PNAS 2015



DMP1(8kb)-Cre; CA(β-catenin)



"Postnatal β-catenin deletion from Dmp1-expressing osteocytes/osteoblasts reduces structural adaptation to loading, but not periosteal load-induced bone formation"

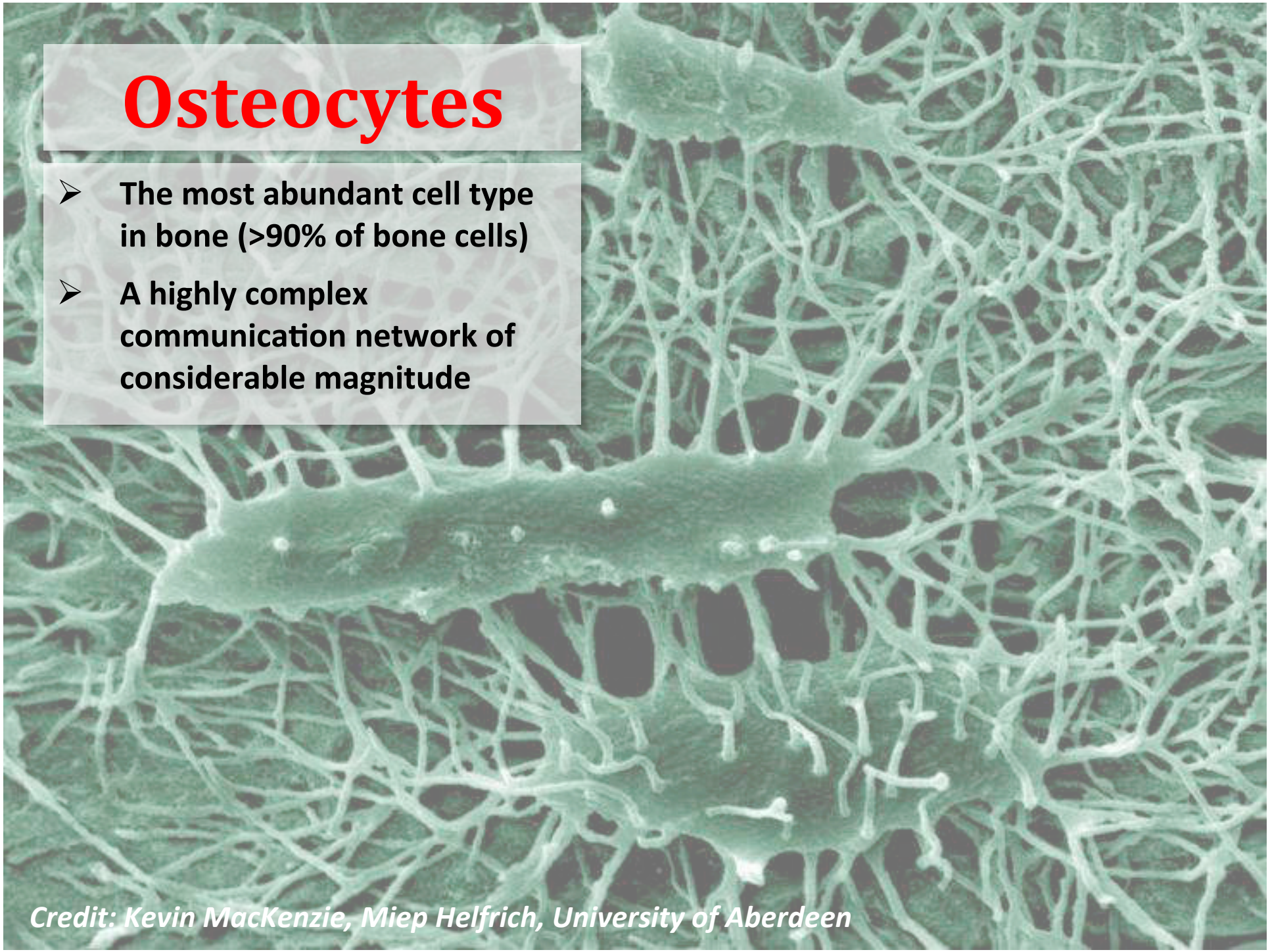
Kyung Shin Kang, Jung Min Hong, Alexander G. Robling

Bone 2016

DMP1(10kb)-CreERt2; β-catenin(flox)

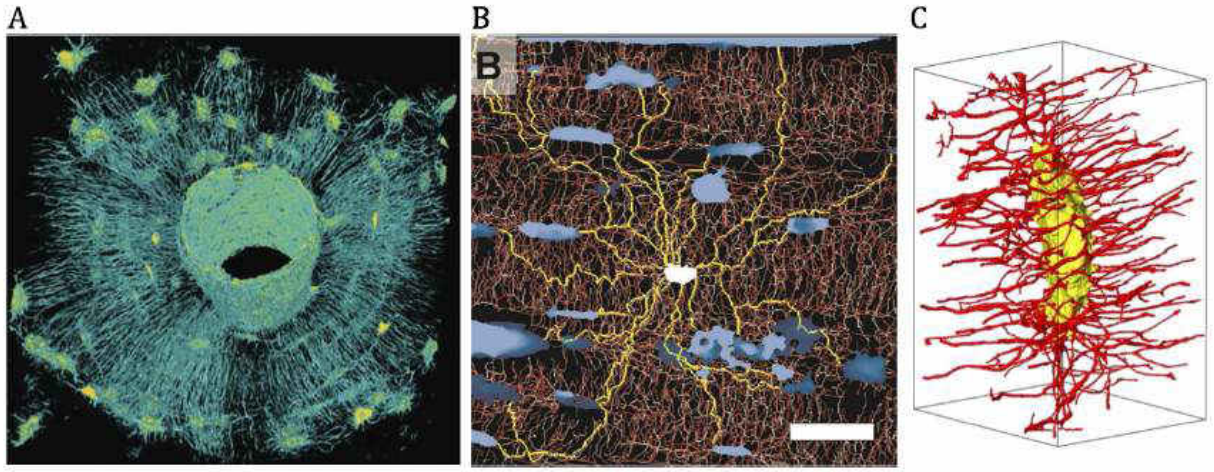
icKO (tamoxifen @ week 17)

Adult-onset deletion of βcat ➔ **reduced bone mass and density** over a 4-wk period **+ Altered response to mechanical loading**


Osteocytes
The most abundant cell type in bone (>90% of bone cells)
A highly complex communication network of considerable magnitude
Credit: Kevin MacKenzie, Miep Helfrich, University of Aberdeen

"Quantifying the osteocyte network in the human skeleton"

Buenzli PR, Sims NA. **Bone** 75:144-150, **2015**



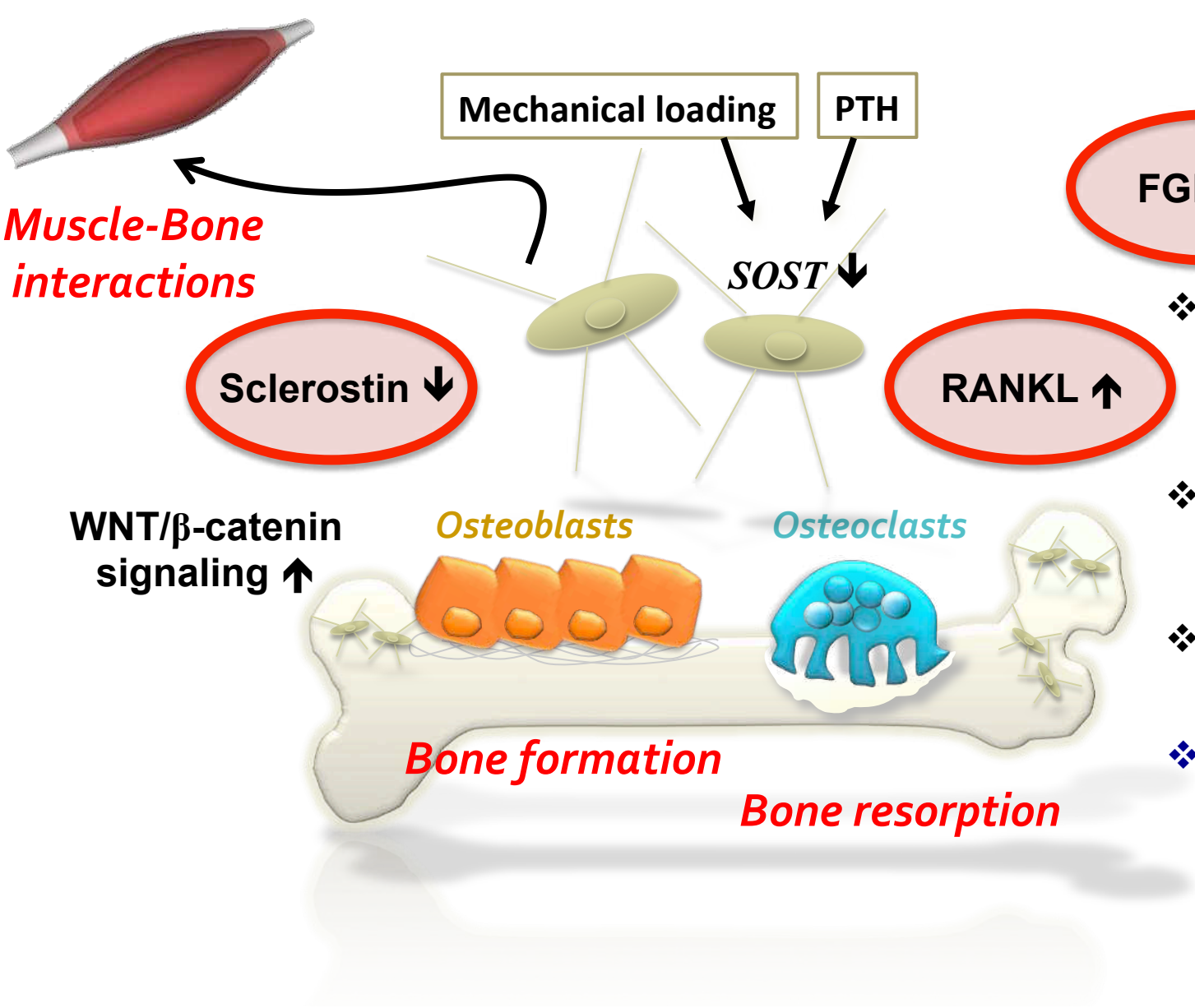
- **Using data of recent papers, obtained with advanced imaging techniques**
  synchrotron X-ray, Nano-CT
- **Using published literature**
  > Parfitt 1983 – now
- **Arythmetic calculations and mathematical models**

e.g.: total # osteocytes (Ots) = total BV x estimated average osteocyte density (# Ots/BV)

Estimations of the magnitude of the osteocyte network in human bone:

	Osteocyte network in bone	Neuronal network in brain
Total # cells in human body	**≈ 42 billion**	**≈ 86 billion**
Total processes length	**≈ 175,000 km**	**≈ 150,000 – 180,000 km**
Total # dendritic projections	≈ 3.7 trillion (≈ 89 per Ot)	
# connections (with other Ots and bone surface cells)	≈ 23.4 trillion (on average 12.7 termini per cell process)	≈ 150 trillion neural cortex synapses

The Multifunctional Osteocyte



Phosphate homeostasis

FGF-23

- Respond to mechanical forces and osteoanabolic stimuli
- Control bone formation and resorption
- Act as endocrine cells

- Produce factors that decrease muscle mass and function with age

The muscle-bone functional unit

Molecular Clocks Symposium (Tuesday)

muscle icKO-Bmal1: Clock genes

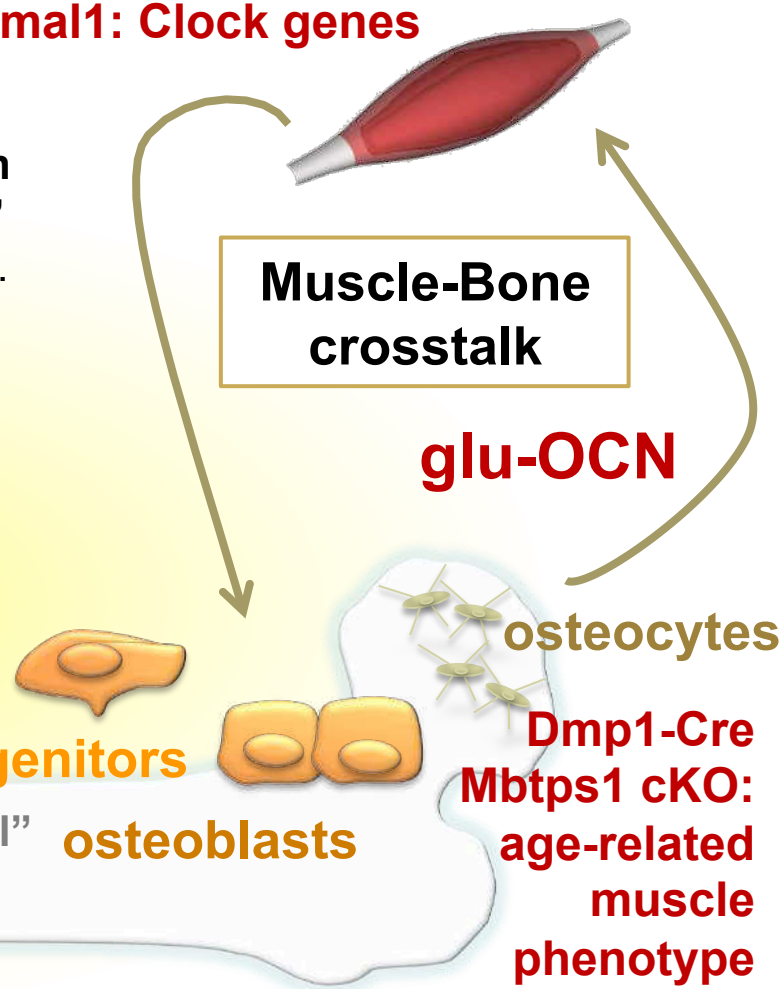
"Deletion of Mbtps1 (Pcsk8, S1p, Ski-1) Gene in Osteocytes Stimulates Soleus Muscle Regeneration and Increased Size and Contractile Force with Age"
Gorski et al. *J Biol Chem*. 2016 Feb 26;291(9):4308-22.

"Deletion of connexin43 in osteoblasts/osteocytes leads to impaired muscle formation in mice"
Shen et al. *J Bone Miner Res*. 2015 Apr;30(4):596-605.

"Intrinsic muscle clock is necessary for musculoskeletal health"
Schroder et al. *J Physiol*. 2015 Dec 15;593(24): 5387-404.

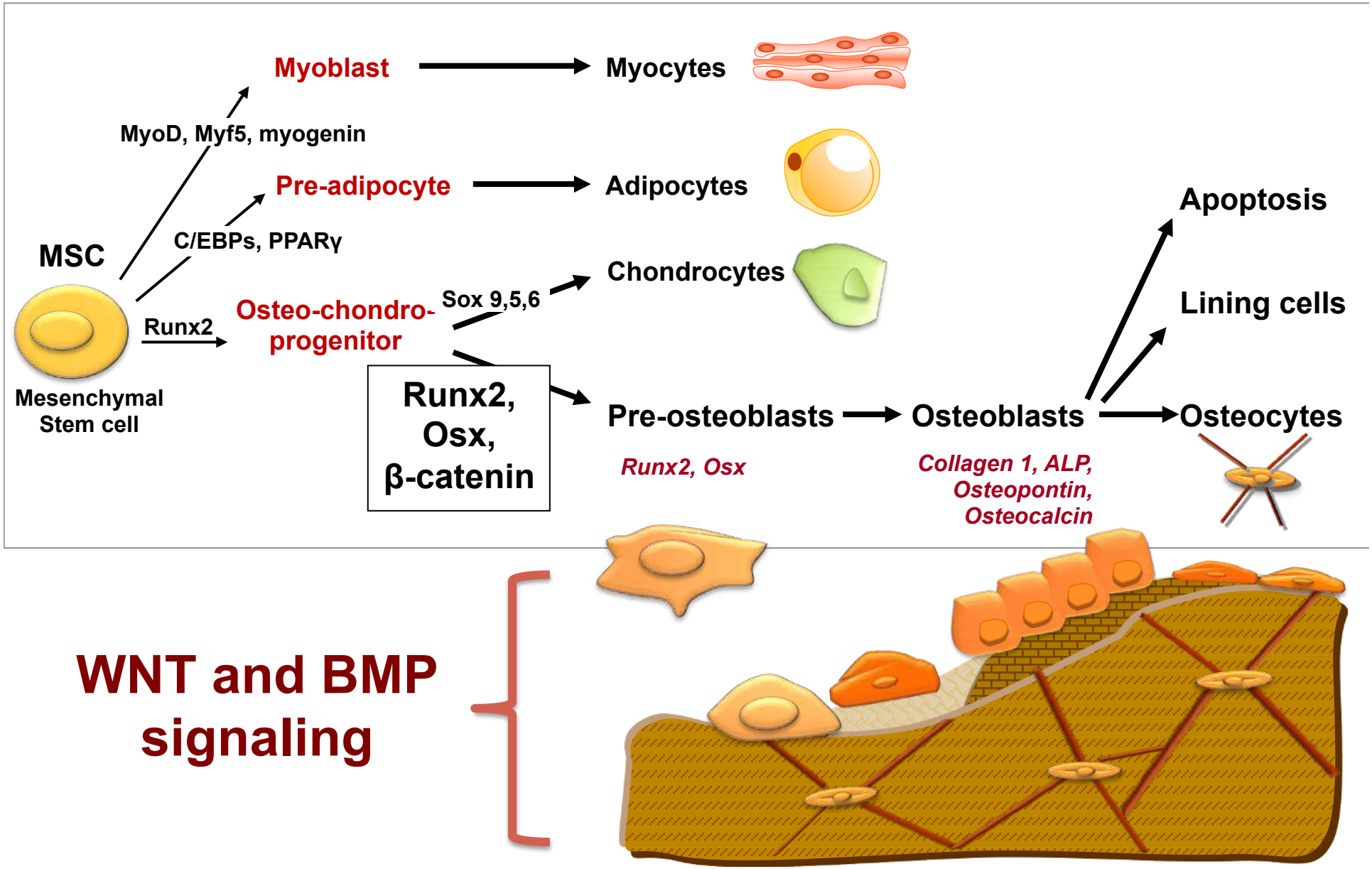
"Bone and muscle: Interactions beyond mechanical"
Brotto and Bonewald. *Bone*. 2015 Nov;80:109-14.

"Muscle and bone, two interconnected tissues"
Tagliaferri et al. *Ageing Res Rev*. 2015 May;21:55-70.



Muscle and Bone Sessions (Monday)

Osteoblast differentiation and bone formation



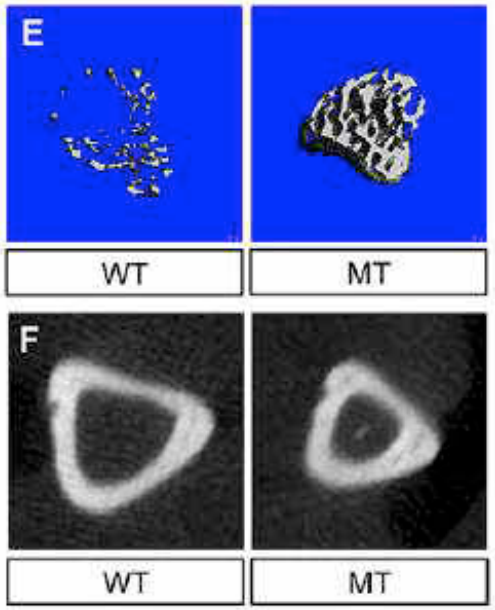
Osteoblast differentiation and bone formation

DMP1-Cre; BMPR1a(flox)

PN Osx-Cre^{dox}; BMPR1a(flox)

"Dual function of Bmpr1a signaling in restricting preosteoblast proliferation and stimulating osteoblast activity in mouse"

Lim et al. Development. 2016



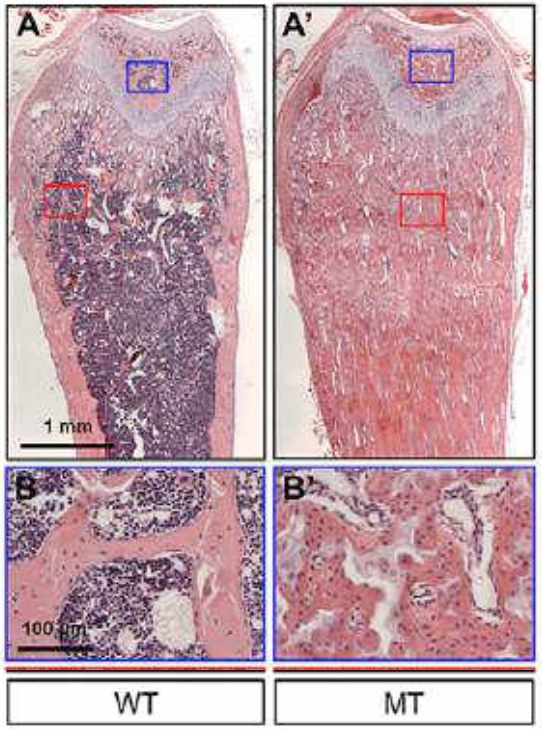
PN Col1-CreERt; BMPR1a(flox)

"Mechanical loading synergistically increases trabecular bone volume and improves mechanical properties in the mouse when BMP signaling is specifically ablated in osteoblasts"

Iura et al. PLoS One. Oct 2015.

"Loss of BMP signaling through BMPR1A in osteoblasts leads to greater collagen cross-link maturation and material-level mechanical properties in mouse femoral trabecular compartments"

Zhang et al. Bone. Apr 2016.



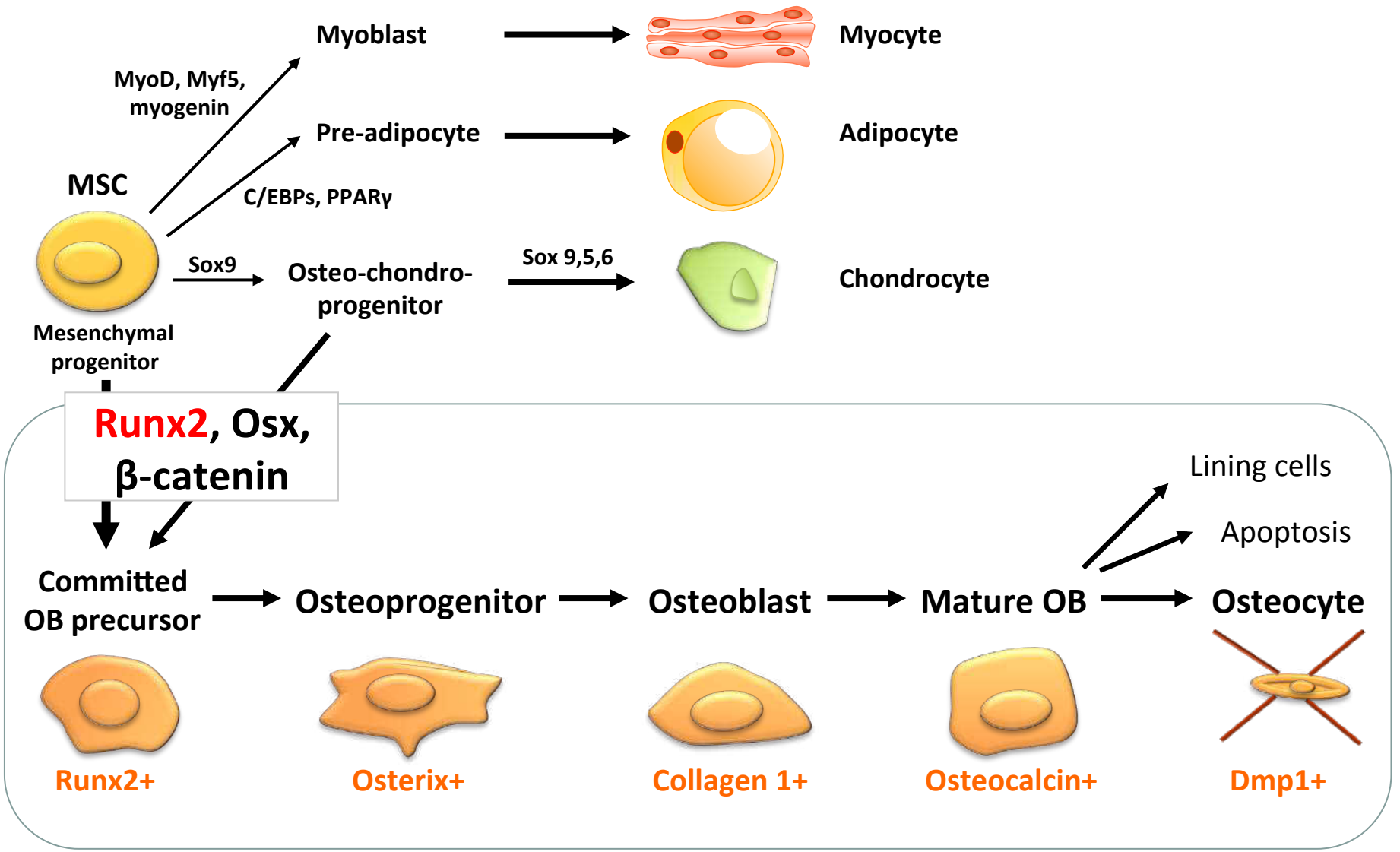
Trabecular bone ↑

 # OBs ↑

SOST ↓

BMP signaling can negatively regulate bone formation

Osteoblast differentiation and bone formation



Cell

Article

Glucose Uptake and Runx2 Synergize to Orchestrate Osteoblast Differentiation and Bone Formation

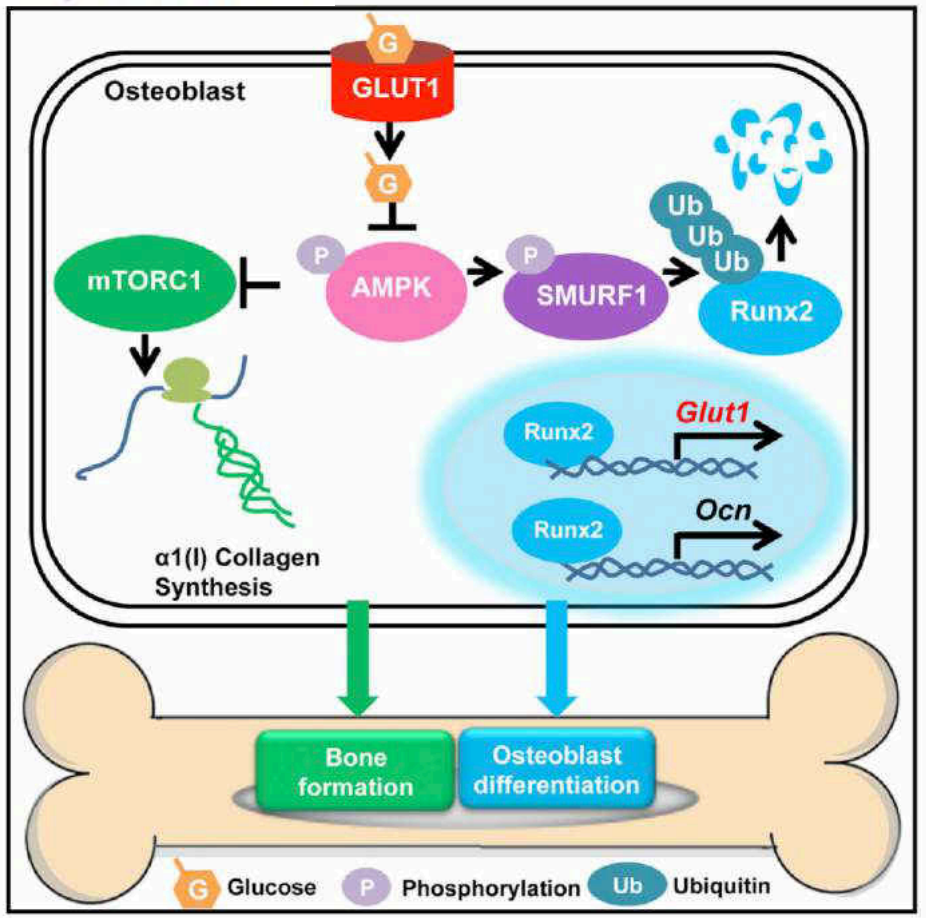
Authors

Jianwen Wei, Junko Shimazu, Munevver P. Makinistoglu, ..., Jeffrey E. Pessin, Eiichi Hinoi, Gerard Karsenty

Highlights

- Osteoblasts are addicted to glucose
- Glucose uptake is needed for osteoblast differentiation and bone formation
- Runx2 is necessary for Glut1 expression in prospective osteoblasts
- Glut1 and Runx2 crosstalk determines osteoblast differentiation and bone formation

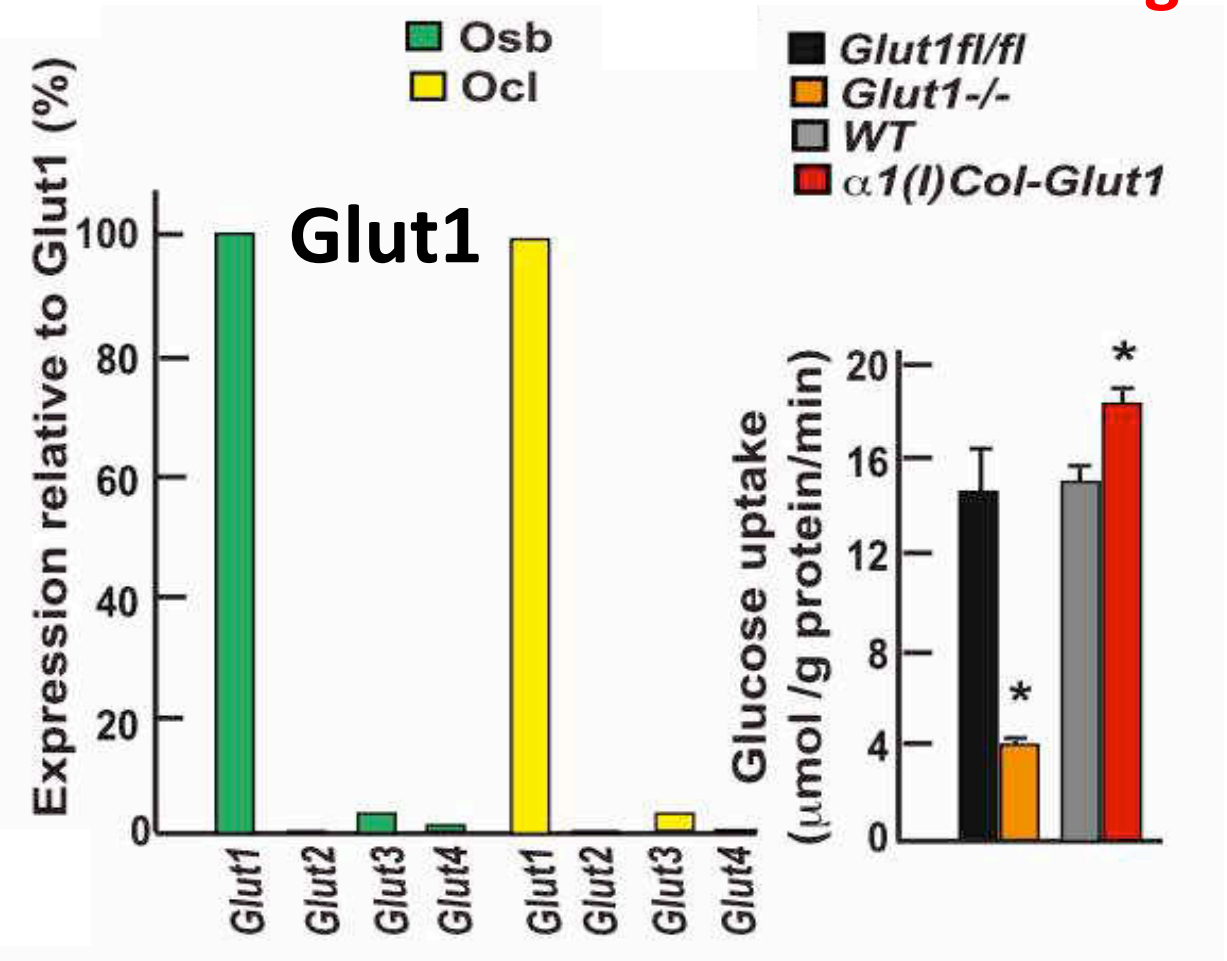
Graphical Abstract

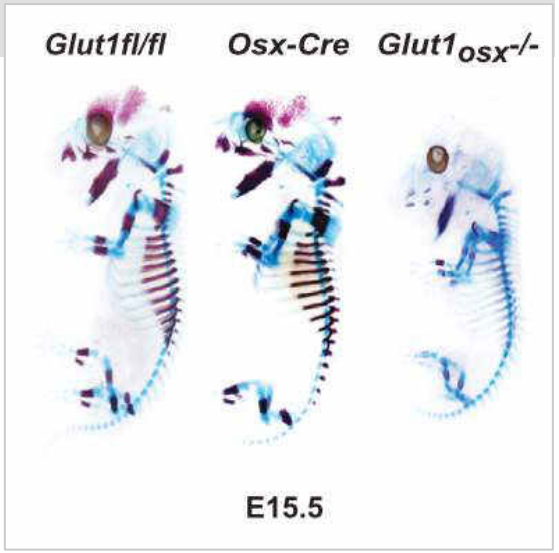


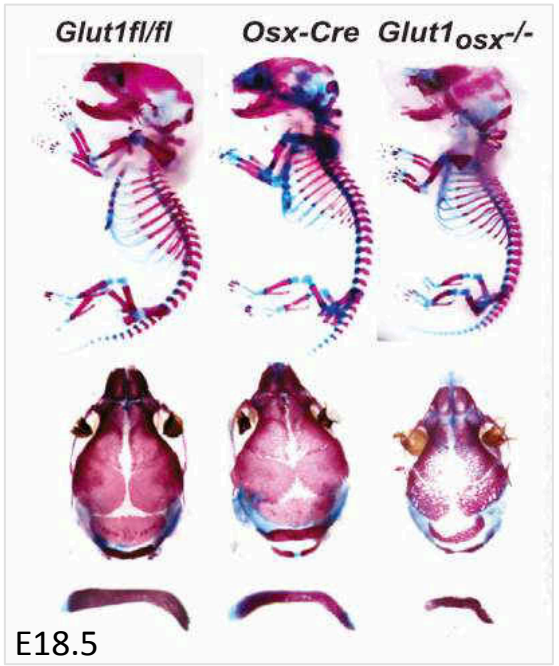
Wei et al., Cell 161:1576-1591, June 2015

Glucose uptake is necessary for osteoblast differentiation and bone formation during development

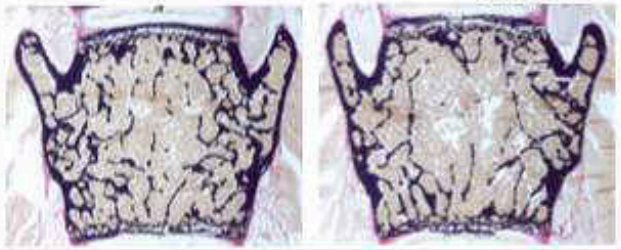
Glut1 cKO
Glut1 Tg







Glucose uptake is necessary for bone formation postnatally



	Glut1fl/fl	Glut1ocn-/-
BV/TV (%)	18.3 ± 0.87	12.78 ± 0.55#
N.Ob/T.Ar	119.19 ± 21.54	66.68 ± 11.45*
MAR (mm/y)	1.68 ± 0.12	1.20 ± 0.13*
BFR/BS ($\mu m^3/\mu m^2/y$)	106.84 ± 13.12	60.78 ± 4.93*
Oc.S/BS	21.24 ± 1.23	23.29 ± 0.99

	WT	α1(I)Col-Glut1
BV/TV (%)	16.91 ± 0.67	19.59 ± 0.88*
N.Ob/T.Ar	124.00 ± 16.65	190.85± 19.36*
MAR (mm/y)	1.38 ± 0.09	1.87 ± 0.13*
BFR/BS ($\mu m^3/\mu m^2/y$)	101.43 ± 12.50	150.66 ± 17.10*
Oc.S/BS	12.65± 1.05	13.49 ± 0.89

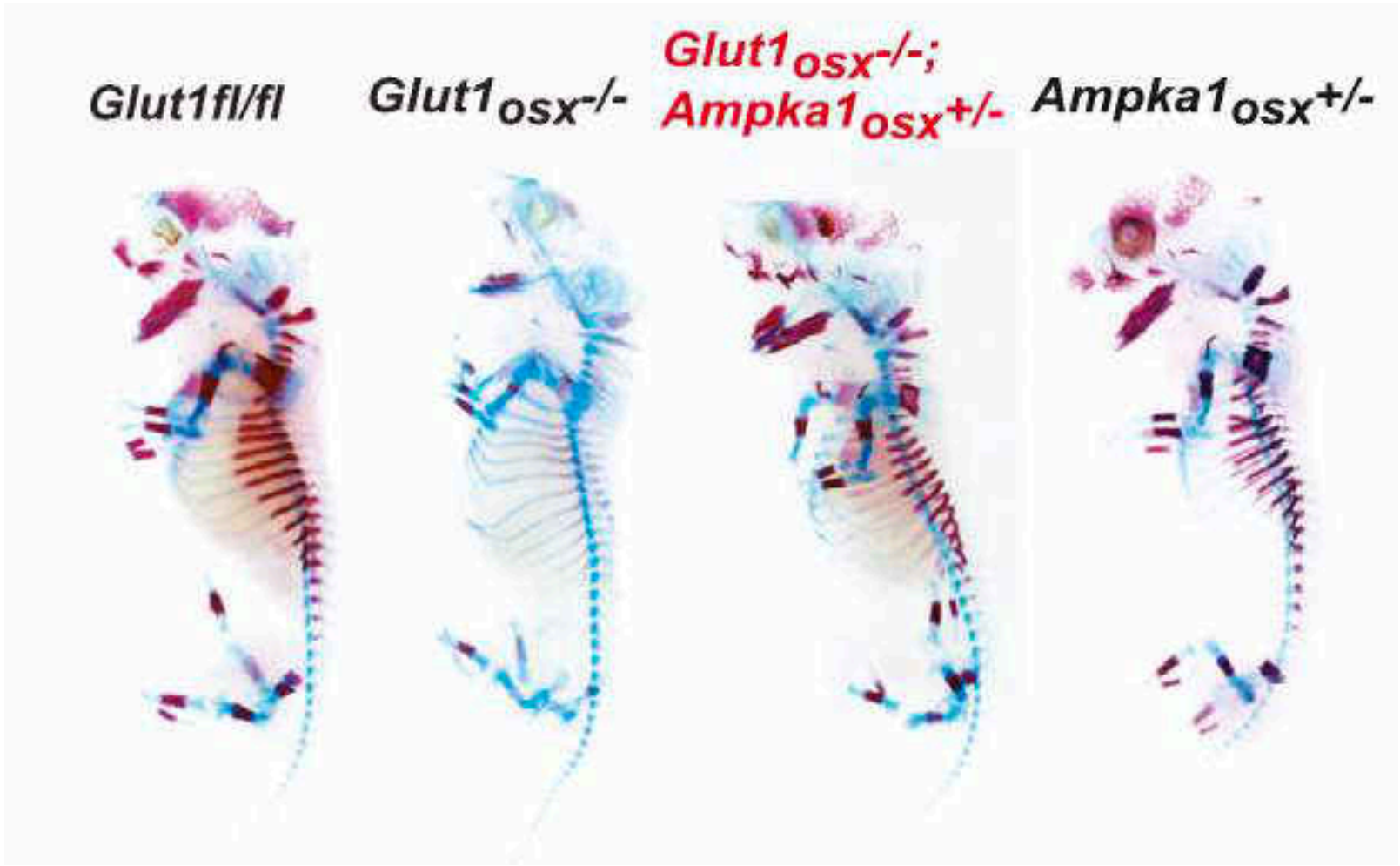
OCN-Cre;Glut1(flox)
cKO

Trab bone ↓
OBs ↓
OB activity ↓

Col1-Glut1
Tg

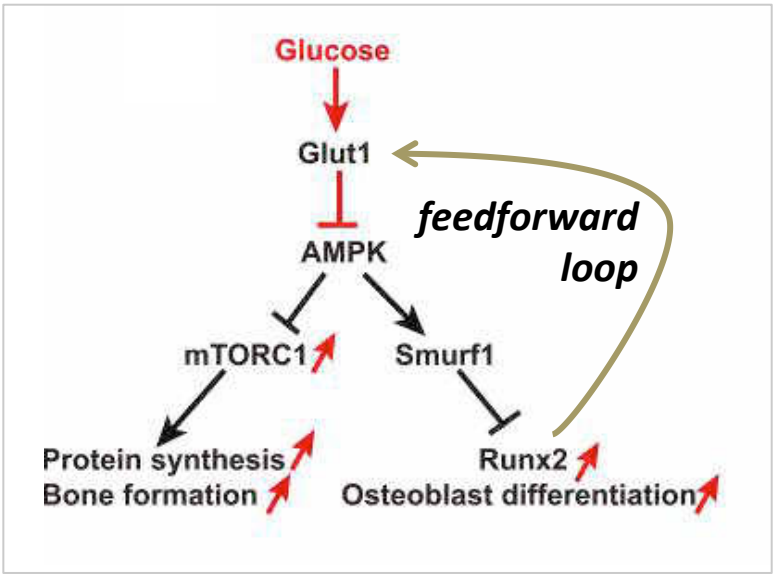
Trab bone ↑
OBs ↑
OB activity ↑

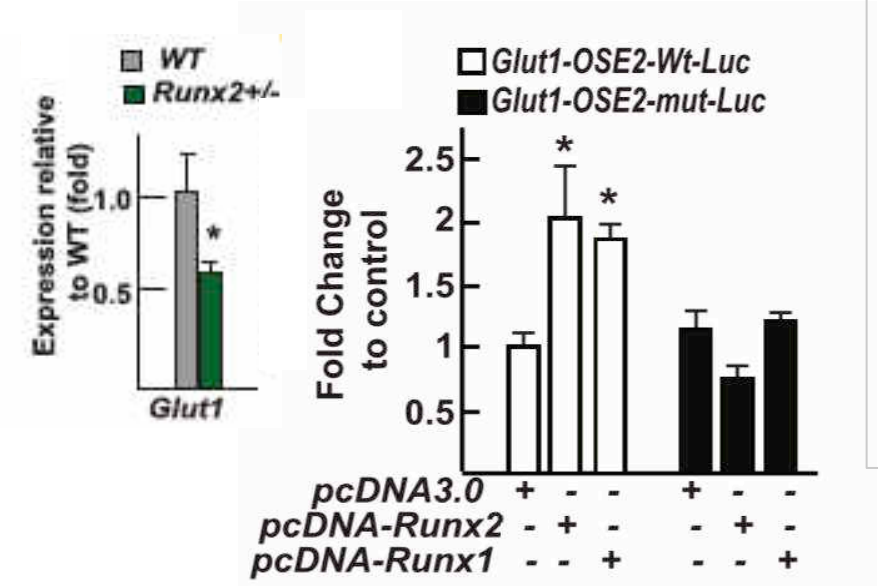
Glut1-deficiency inhibits osteoblast differentiation and bone formation by increasing AMPK

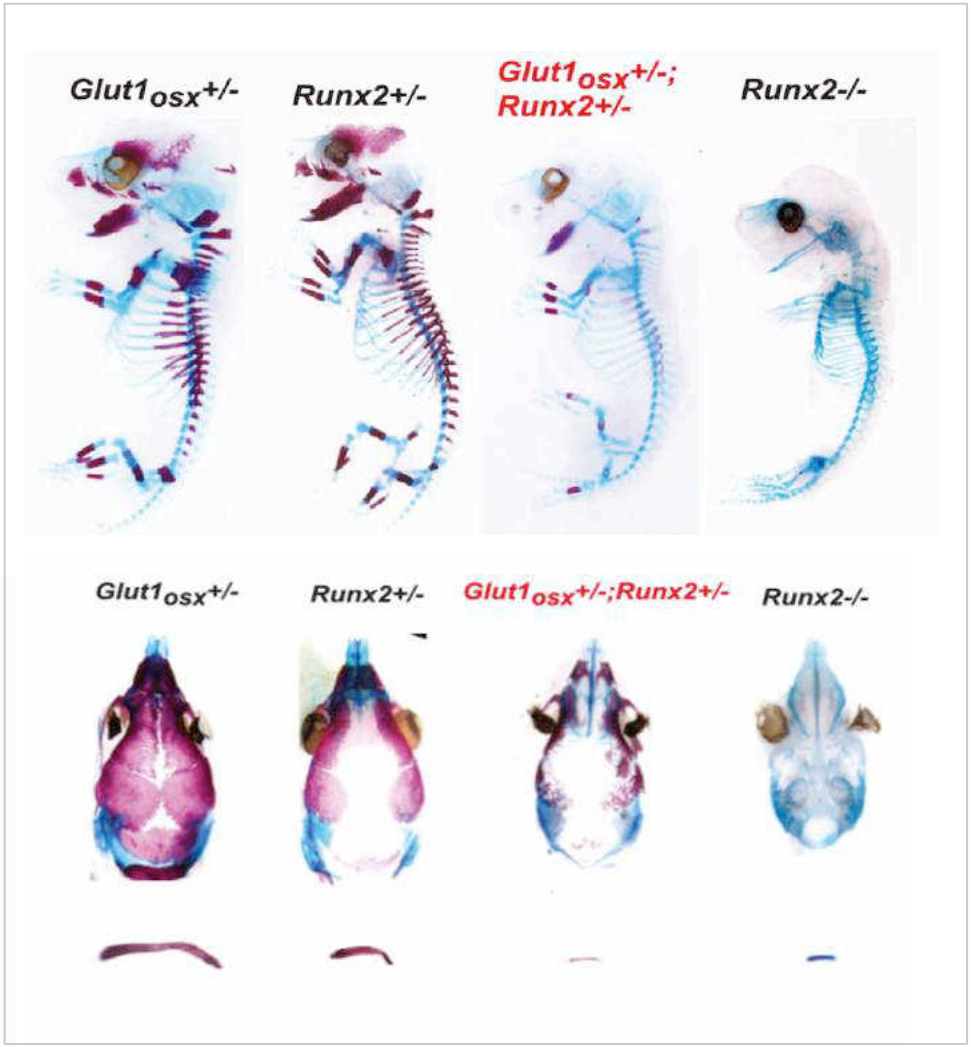


Wei et al., Cell 161:1576-1591, June 2015

Crosstalk between Runx2 and Glut1 coordinates osteoblast differentiation and bone formation







Osteoblast cellular glucose and energy metabolism

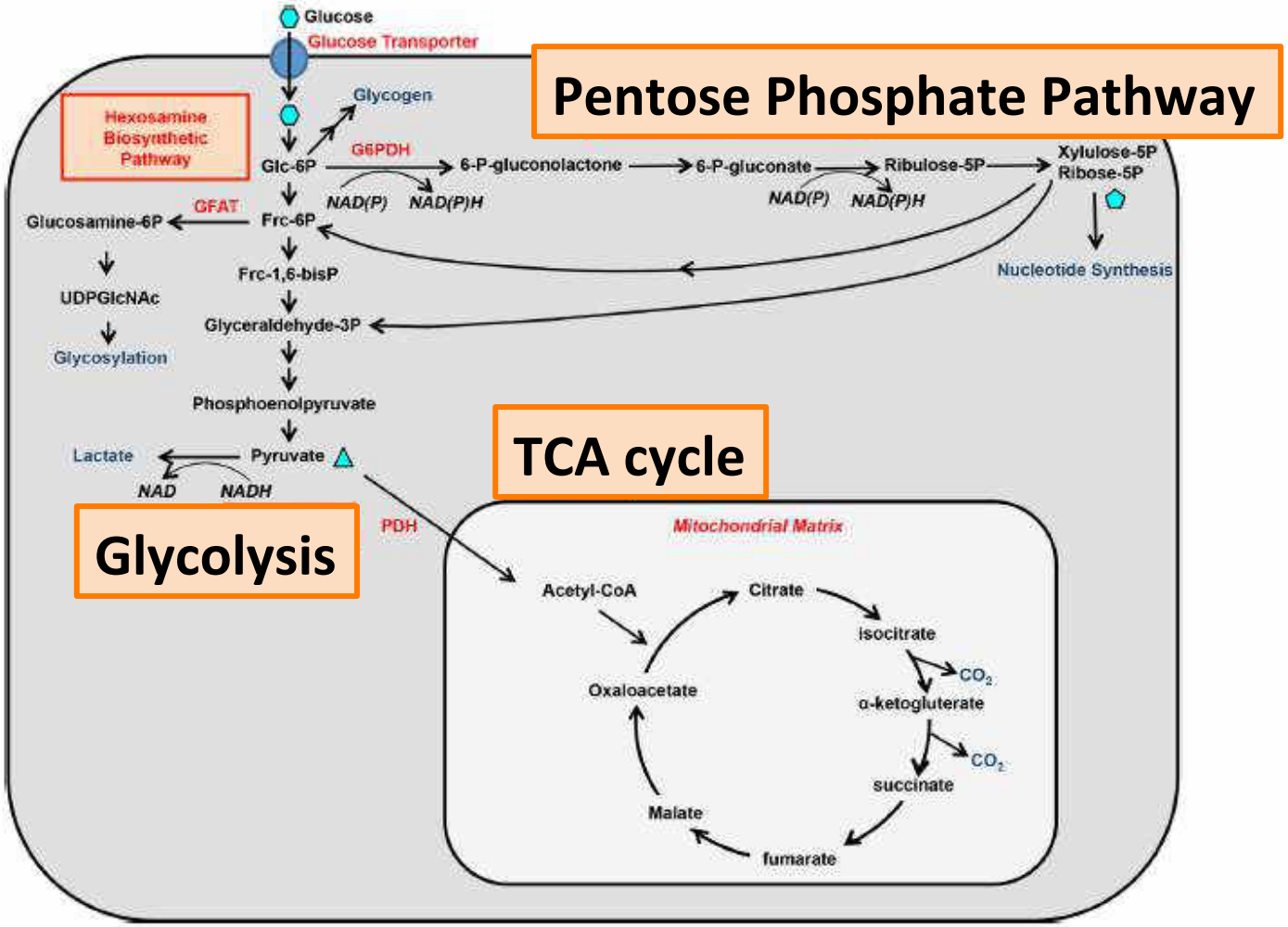
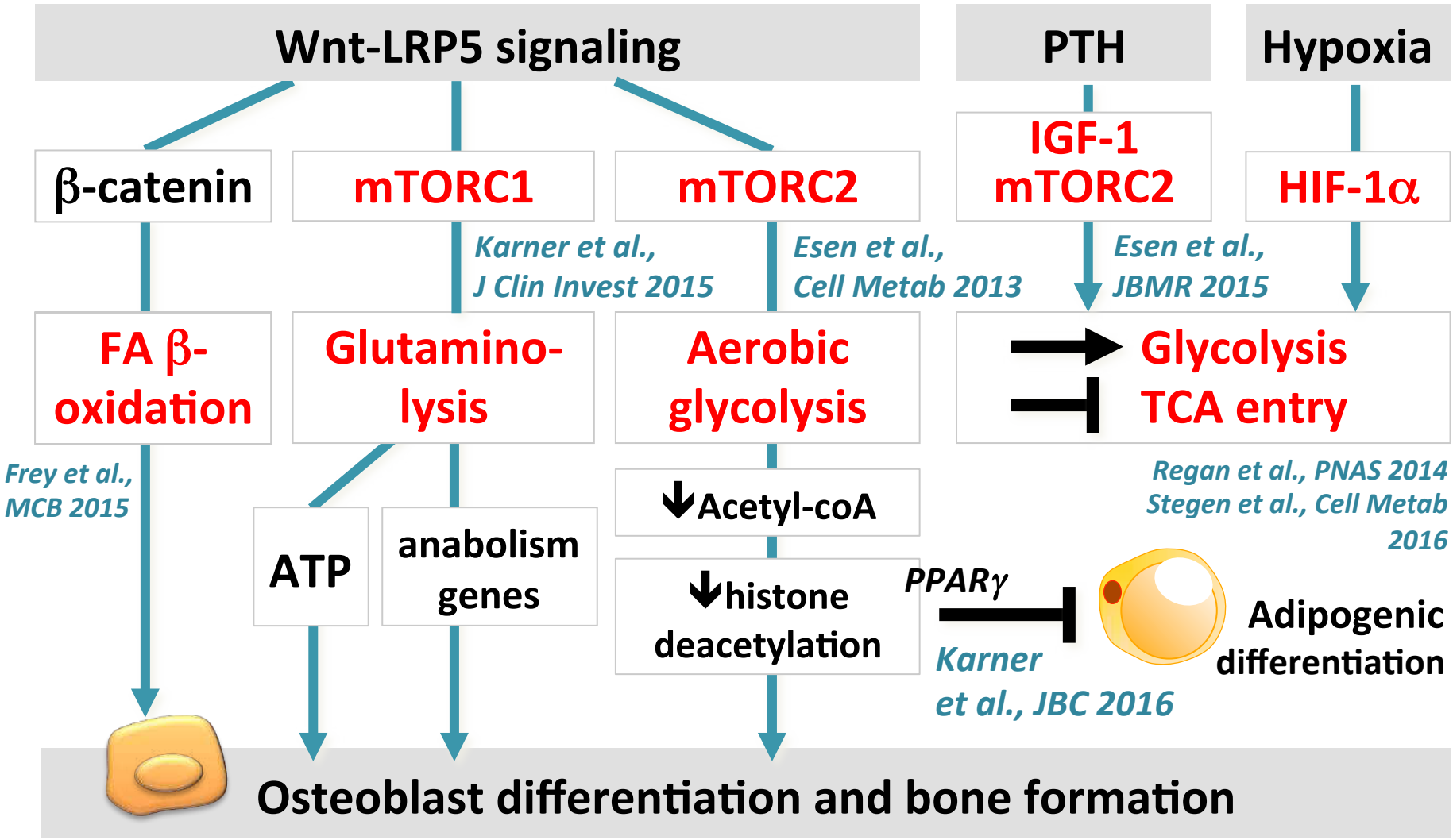


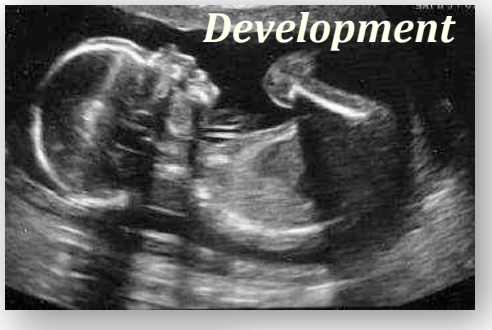
Fig. 1 Metabolic fates of glucose in mammalian cells. G6PDH: Glucose-6-phosphate dehydrogenase. GFAT: Glutamine fructose-6-phosphate amidotransferase. *PDH* Pyruvate dehydrogenase complex

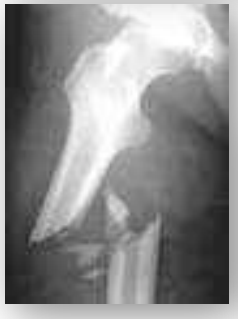
Esen E, Long F. "Aerobic glycolysis in osteoblasts"
Curr Osteoporos Rep. 12:433-438. Dec 2014

Osteoblast energy metabolism



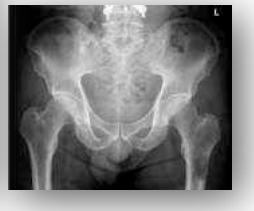
Bone vascularization



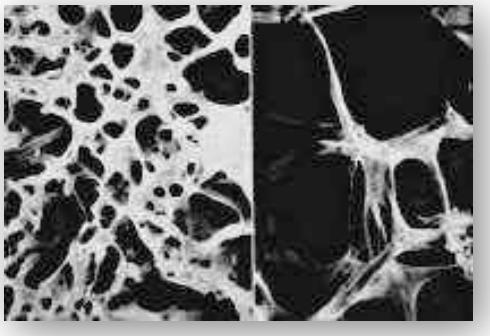


Fracture

Metastasis



Normal Osteoporosis



perivascular mesenchymal (osteo-)progenitors

blood vessels

HSC

endothelial cells

bone marrow

hematopoiesis

osteoclast precursors

HSC

osteoblasts

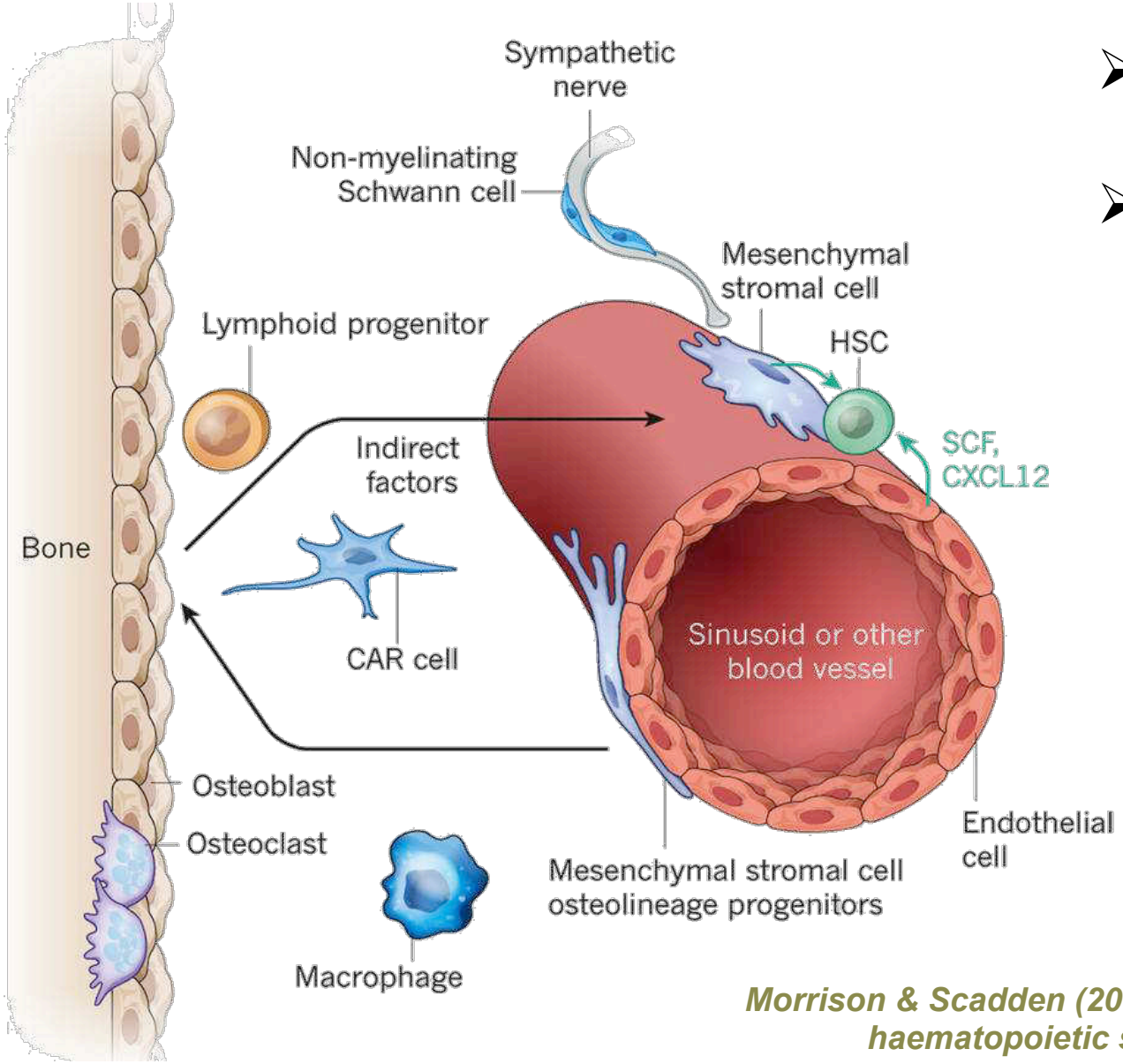
osteoclasts

osteogenesis

bone resorption

Role and regulation of vascularization processes in endochondral bones.
Maes C., Calcif Tissue Int. 2013

Perivascular mesenchymal progenitor populations contain hematopoiesis-supportive cells in mice

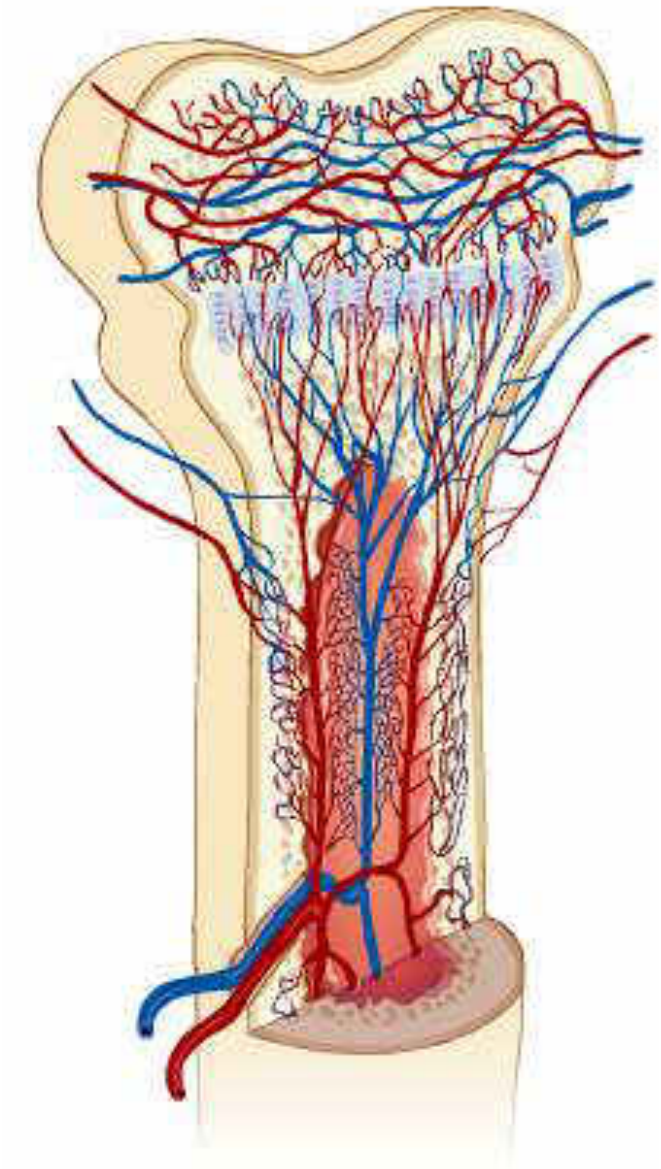


- **HSC support in the perivascular niche**
- **Multipotent: adipo-/osteogenic**

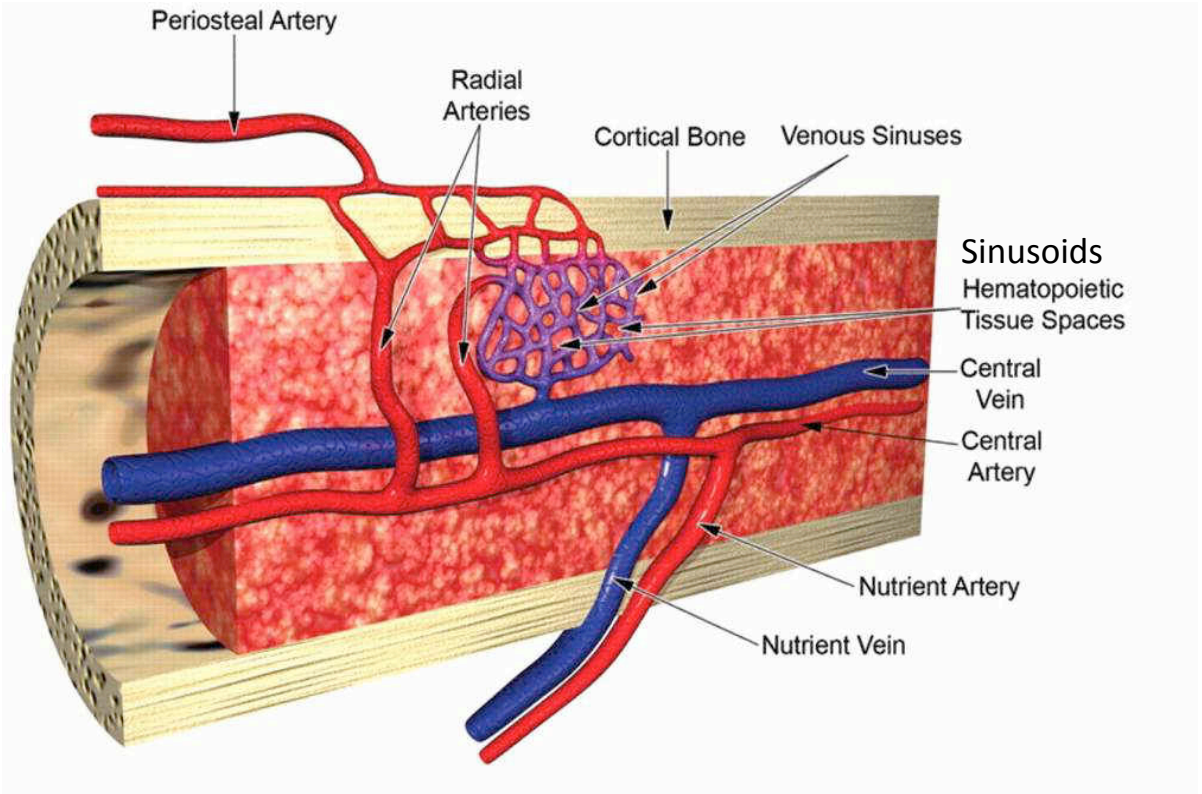
  - **CAR cells ($CXCL12^{high}$)**
  - **$Nestin^{+}$ cells**
  - **$LepR^{+}$ cells**
  - **$PDGFR\alpha/\beta^{+}$ cells**
  - **Osx+ cells**

Morrison & Scadden (2014). The bone marrow niche for haematopoietic stem cells. Nature 505, 327–334

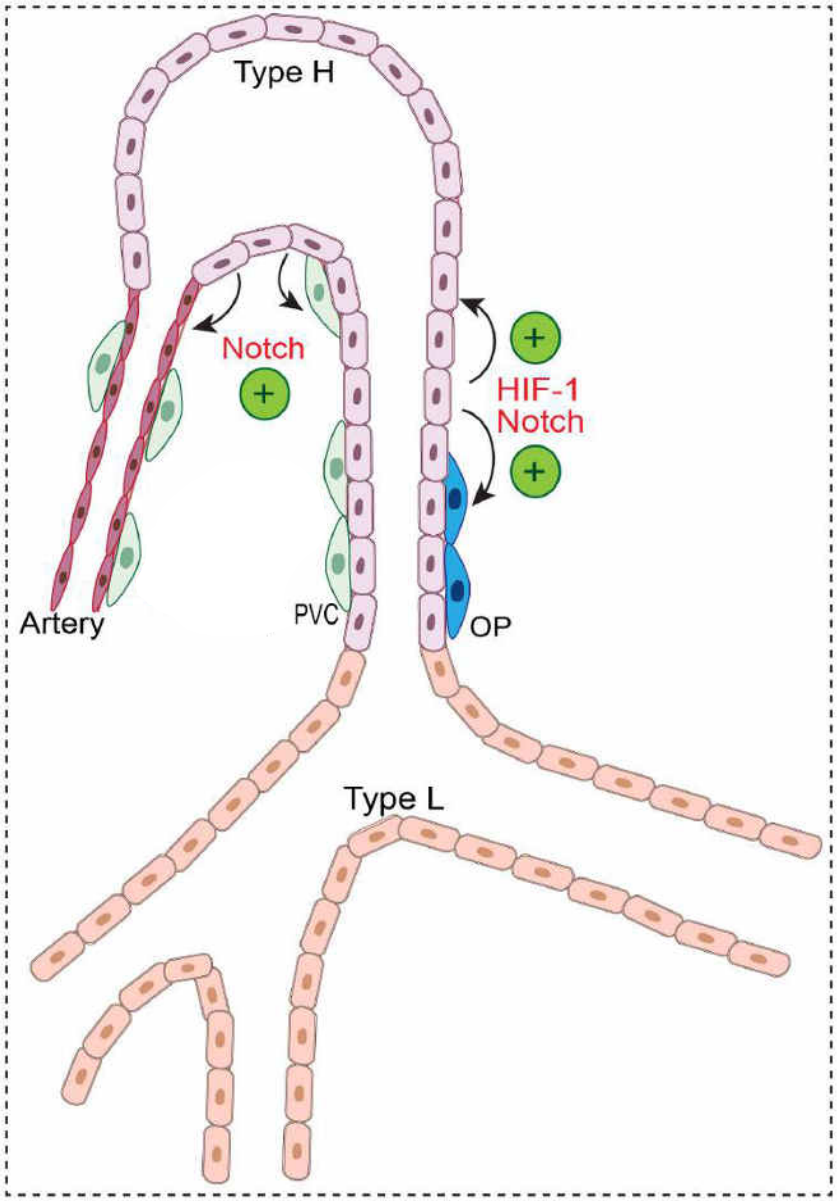
New work on the Bone Vascular System and Hematopoiesis



➢ **The specific location of these perivascular HSC niches remains controversial**



Skeletal Vascular System



Type H capillaries ($CD31^{hi}$ $Emcn^{hi}$):

- Connect to arterioles
- Are surrounded by osteoprogenitors
- Release factors promoting osteogenesis

< 2% of bone ECs

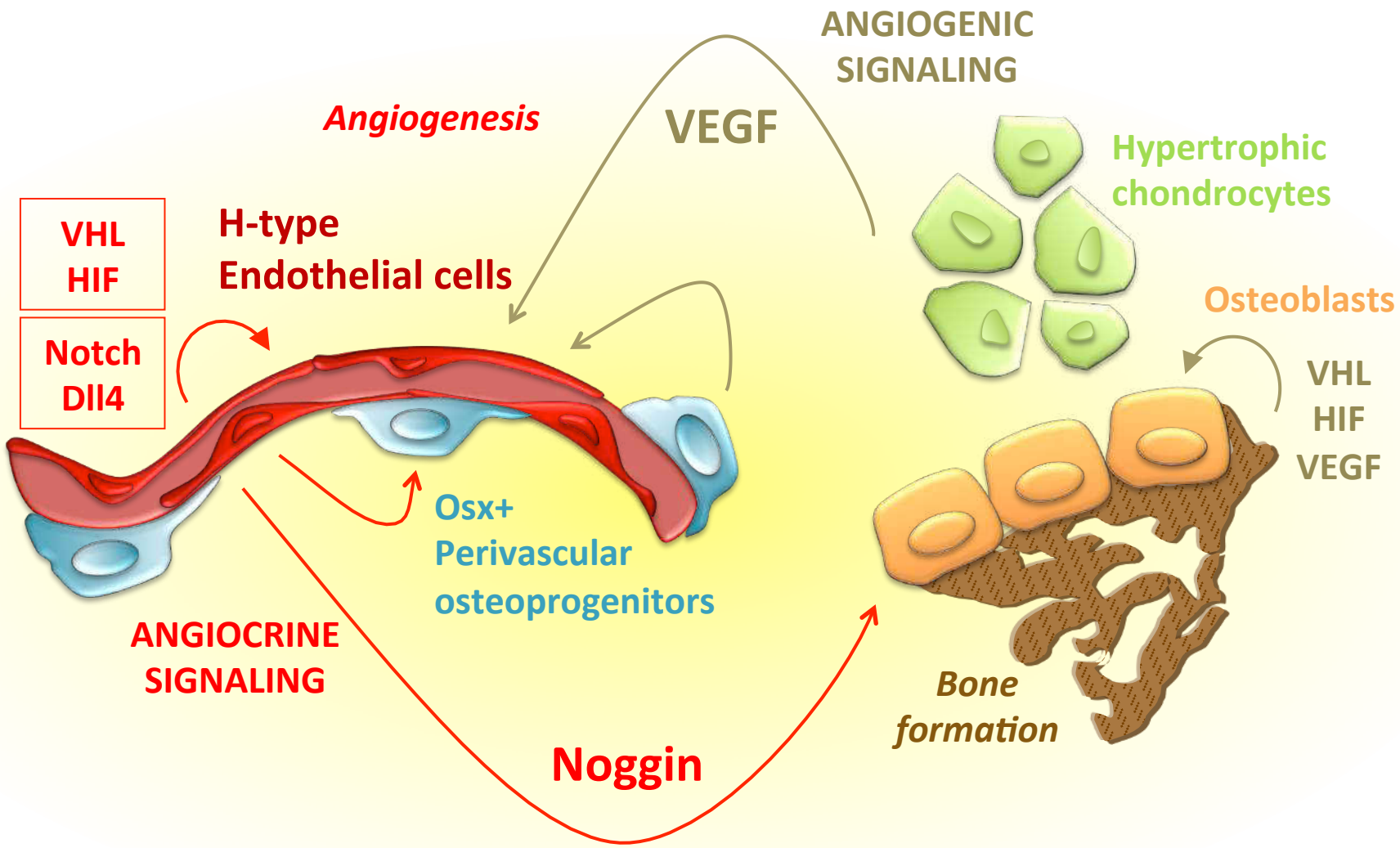
Type L ($CD31^{lo}$ $Emcn^{lo}$) vessels:

- Correspond to BM sinusoids
- Lack arteriolar connections
- Lack osteoprogenitor association

Endothelial Notch activity promotes angiogenesis and osteogenesis in bone. Ramasamy SK, Kusumbe AP, Wang L, Adams RH. Nature. 2014; 507(7492):376-80.

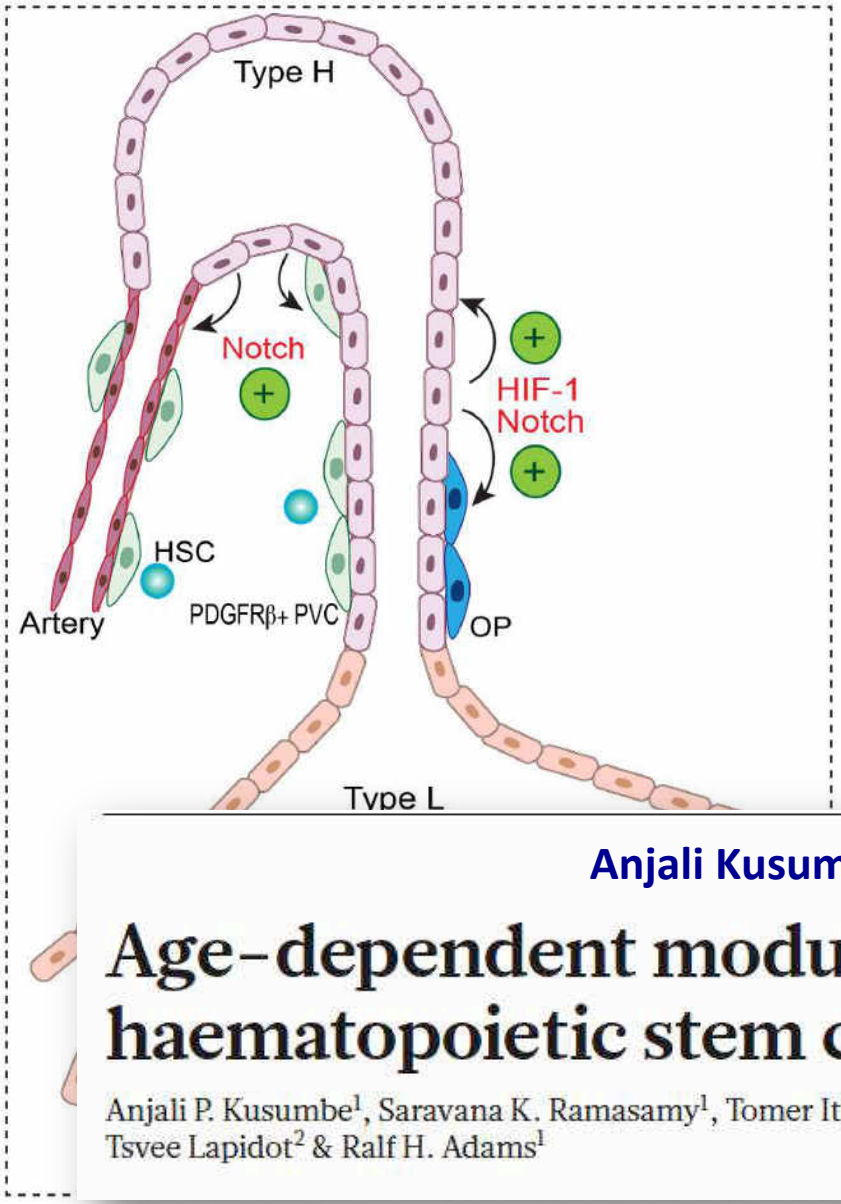
Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. Kusumbe AP, Ramasamy SK, Adams RH. Nature. 2014;507(7492):323-8.

Angiogenic-Osteogenic Coupling



Angiogenic-osteogenic coupling: the endothelial perspective.
Maes C, Clemens TL. Bonekey Rep. 2014 Oct 15;3:578.

HSC niche expansion by endothelial Notch signaling



Type H capillaries (CD31^{hi} Emcn^{hi}):

- Connect to arterioles
- Are surrounded by **PDGFRβ+ perivascular cells (PVCs)**
- Release factors promoting osteogenesis **and hematopoiesis**
- **Identify a vascular niche for HSCs**

Decreased during ageing

Increased by activation of endothelial Notch signaling

Anjali Kusumbe et al., Nature. 2016 Apr 21;532(7599):380-4

Age-dependent modulation of vascular niches for haematopoietic stem cells

Anjali P. Kusumbe[1], Saravana K. Ramasamy[1], Tomer Itkin[2], Maarja Andaloussi Mäe[3], Urs H. Langen[1], Christer Betsholtz[3,4] Tsvee Lapidot[2] & Ralf H. Adams[1]

Skeletal Vascular System and Hematopoiesis

LETTER

Nature. 2015 Oct 1;526(7571):126-30

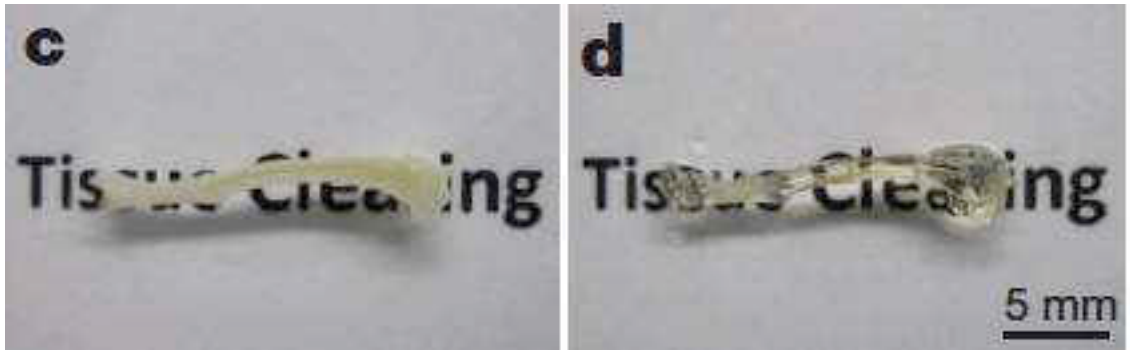
doi:10.1038/nature15250

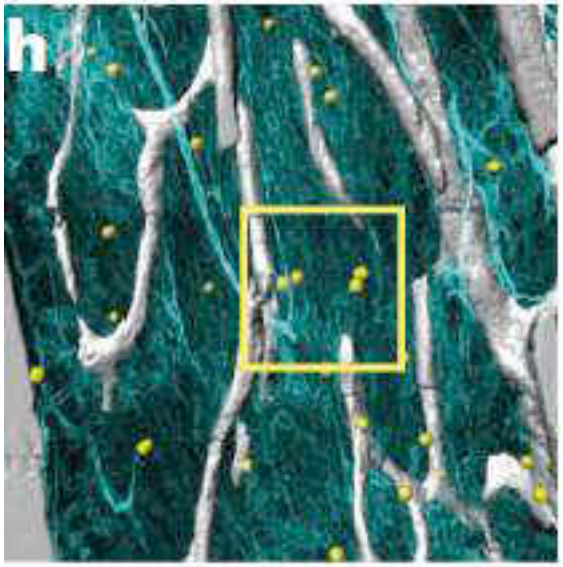
Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal

Melih Acar[2], Kiranmai S. Kocherlakota[1,2]*, Malea M. Murphy[2]*, James G. Peyer[2]*, Hideyuki Oguro[2], Christopher N. Inra[2], Christabel Jaiyeola[2], Zhiyu Zhao[2], Katherine Luby-Phelps[3] & Sean J. Morrison[1,2]

α-catulin-GFP knockin mouse

(marks 0.02% of BM hematopoietic cells, incl. most HSCs (*α-catulin*-GFP+;c-kit+))

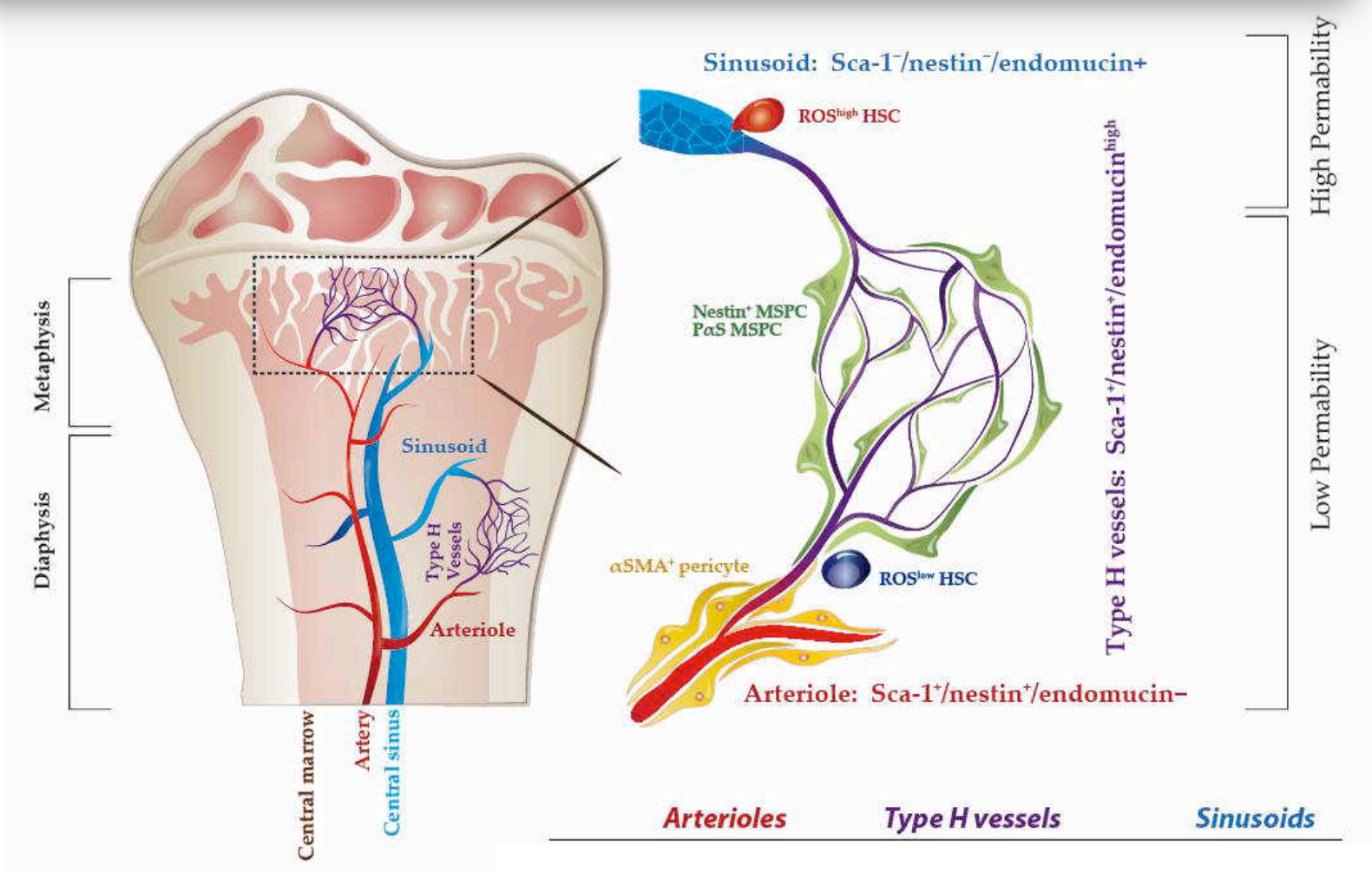




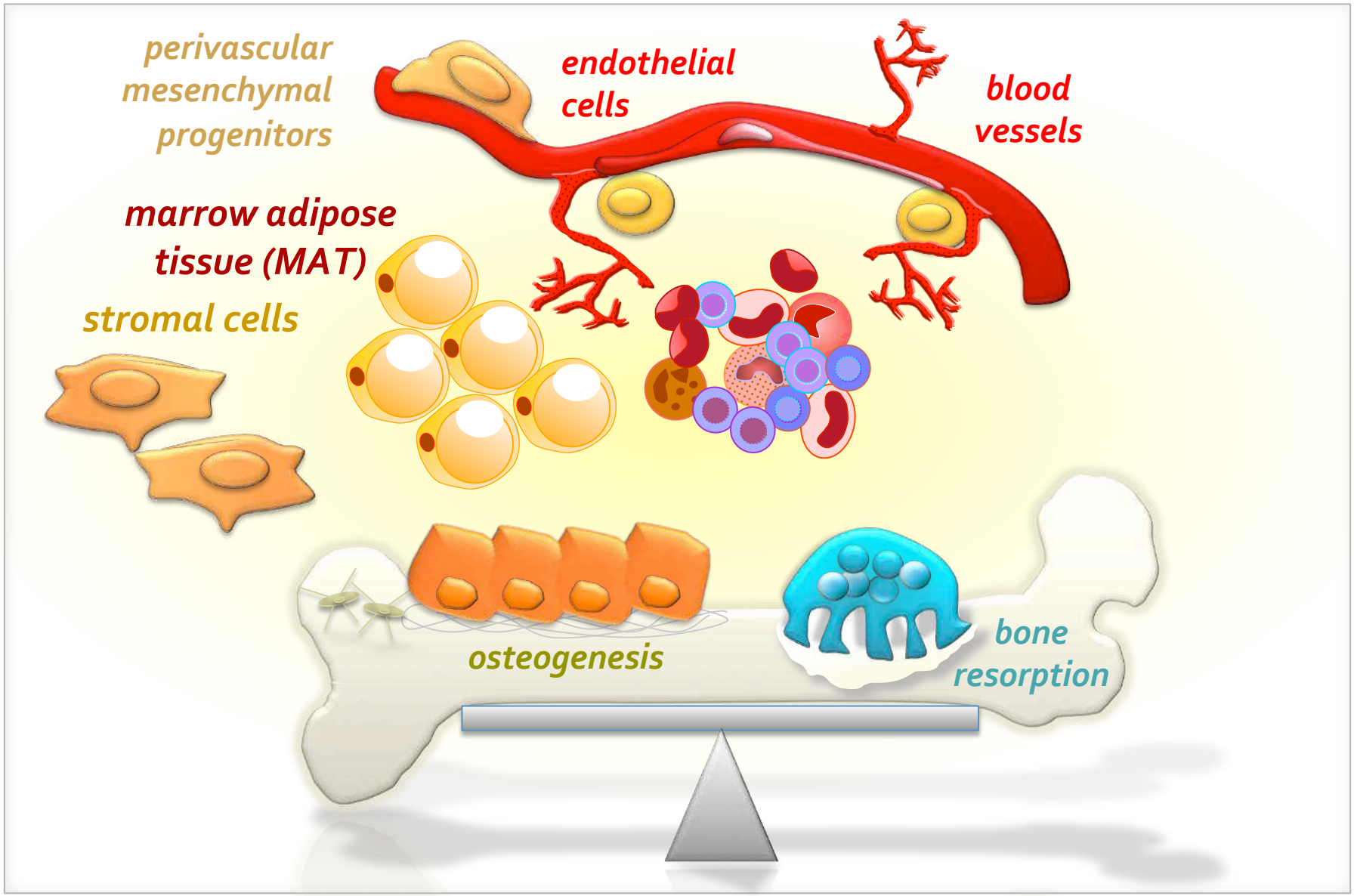
Itkin et al., Nature. 2016 Apr 21;532(7599):323-8

Distinct bone marrow blood vessels differentially regulate haematopoiesis

Tomer Itkin[1], Shiri Gur-Cohen[1], Joel A. Spencer[2,3], Amir Schajnovitz[4,5,6], Saravana K. Ramasamy[7], Anjali P. Kusumbe[7], Guy Ledergor[1,8], Yookyung Jung[2,3], Idan Milo[1], Michael G. Poulos[9], Alexander Kalinkovich[1], Aya Ludin[1], Orit Kollet[1], Guy Shakhar[1], Jason M. Butler[9], Shahin Rafii[9], Ralf H. Adams[7], David T. Scadden[4,5,6], Charles P. Lin[2,3] & Tsvee Lapidot[1]



Increased insights in the interactions between bone cells and their microenvironment

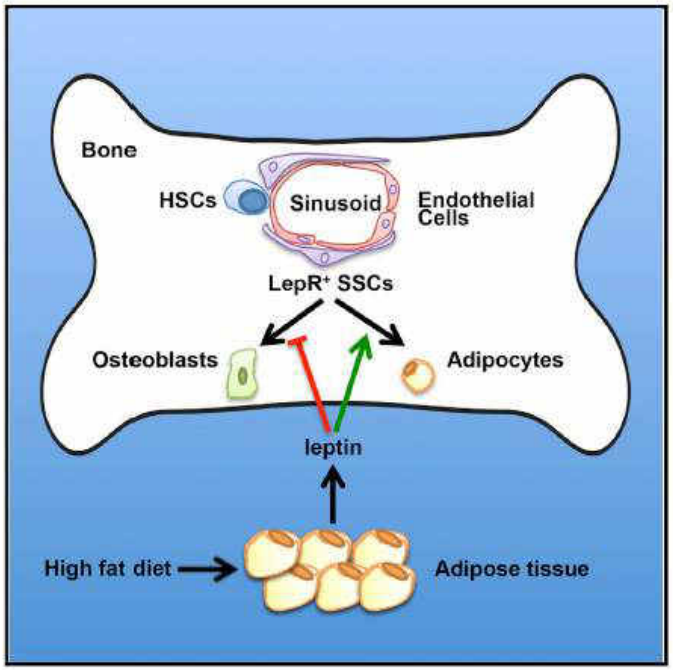


Article

Cell Stem Cell

Leptin Receptor Promotes Adipogenesis and Reduces Osteogenesis by Regulating Mesenchymal Stromal Cells in Adult Bone Marrow

Graphical Abstract



Authors

Rui Yue, Bo O. Zhou, Issei S. Shimada, Zhiyu Zhao, Sean J. Morrison

Correspondence

sean.morrison@utsouthwestern.edu

In Brief

A fundamental question concerns how stem cells are regulated by nutrition and systemic energy homeostasis. Morrison and colleagues demonstrate that leptin receptor acts within skeletal stem cells in the bone marrow as a sensor of systemic energy homeostasis, promoting adipogenesis and inhibiting osteogenesis in response to diet and adiposity.

Yue et al., Cell Stem Cell, June 2016

HFD

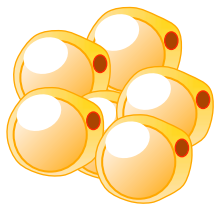
↓

Leptin

LepR+ bipotent progenitors

cell fate

osteoblasts



marrow adipocytes

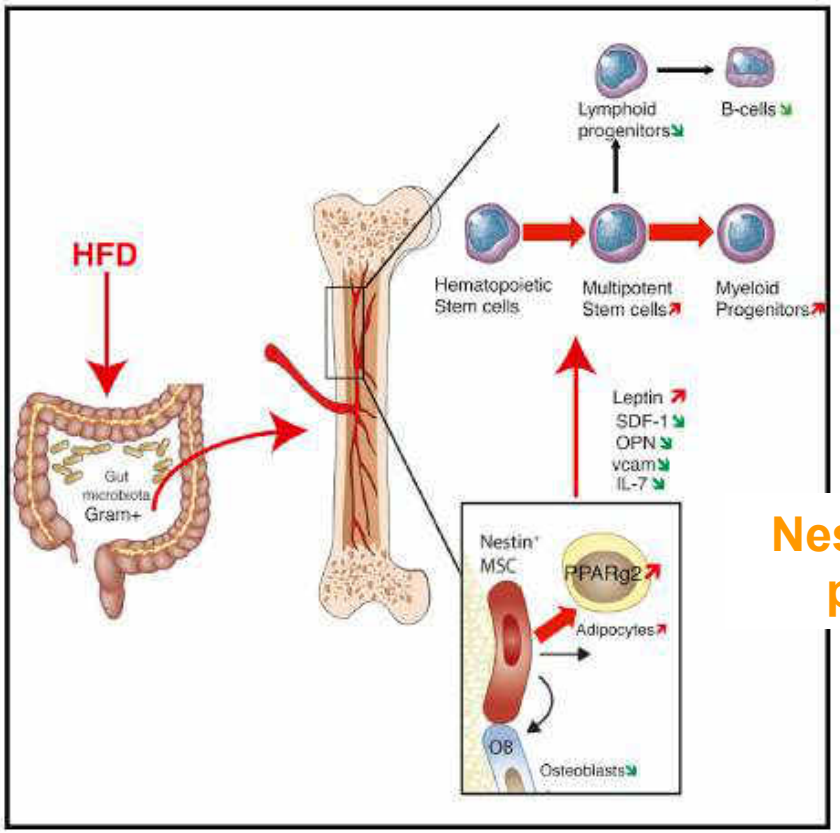
LepR in MSCs acts as a sensor of systemic energy homeostasis

Luo et al., Cell Metabl, Nov 2015

Cell Metabolism

Microbiota from Obese Mice Regulate Hematopoietic Stem Cell Differentiation by Altering the Bone Niche

Graphical Abstract



Authors

Yubin Luo, Guang-Liang Chen, Nicole Hannemann, ..., Stefan Wirtz, Georg Schett, Aline Bozec

HFD

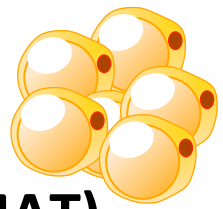
↓

Gut microbiota

Nestin+ bipotent progenitors

cell fate

osteoblasts



marrow adipocytes

HFD alters the BM niche (increased MAT) and alters HSC differentiation

The Journal of Clinical Investigation

J Clin Invest. 2016 Apr 25. pii: 86062

Sex steroid deficiency–associated bone loss is microbiota dependent and prevented by probiotics

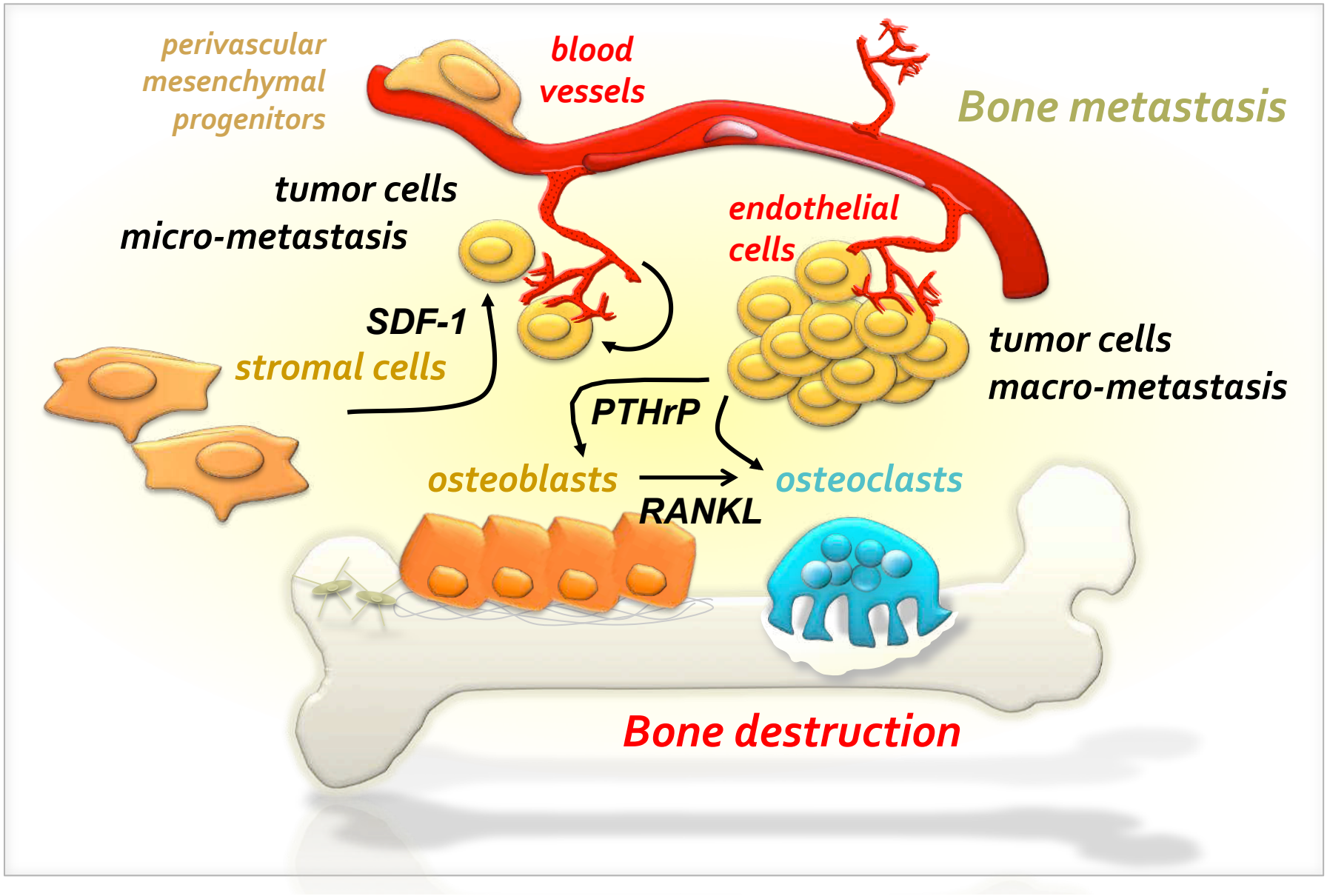
Jau-Yi Li,[1] Benoit Chassaing,[2] Abdul Malik Tyagi,[1] Chiara Vaccaro,[1] Tao Luo,[1] Jonathan Adams,[1] Trevor M. Darby,[3] M. Neale Weitzmann,[1,4] Jennifer G. Mulle,[5] Andrew T. Gewirtz,[2] Rheinallt M. Jones,[3] and Roberto Pacifici[1,6]

[1]Division of Endocrinology, Metabolism and Lipids, Department of Medicine, Emory University, Atlanta, Georgia, USA. [2]Center for Inflammation, Immunity and Infection, Institute for Biomedical Sciences, Georgia State University, Atlanta, Georgia, USA. [3]Department of Pediatrics, Emory University, Atlanta, Georgia, USA. [4]Atlanta Department of Veterans Affairs Medical Center, Decatur, Georgia, USA. [5]Department of Epidemiology, Rollins School of Public Health, and [6]Immunology and Molecular Pathogenesis Program, Emory University, Atlanta, Georgia, USA.

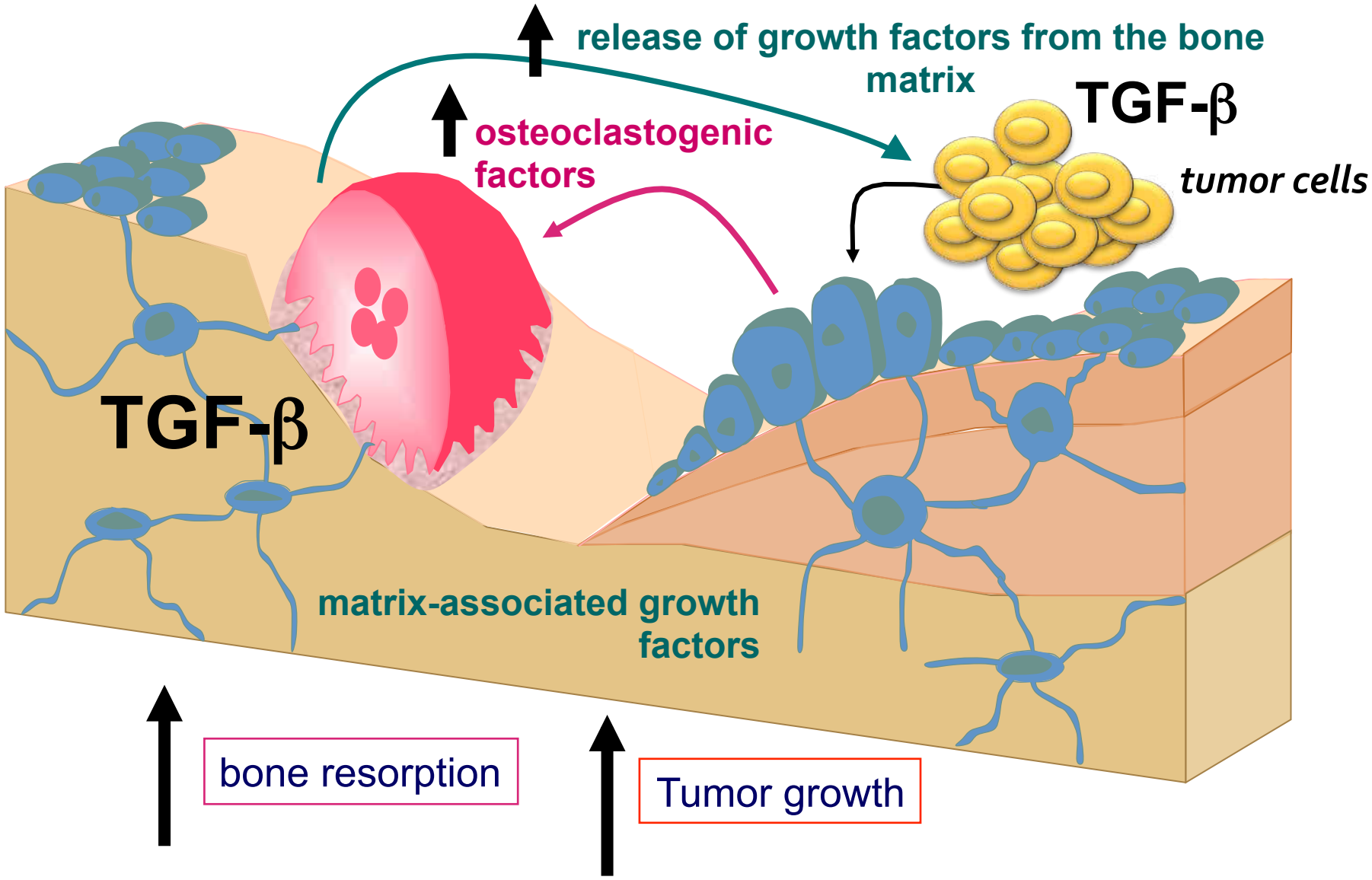
A eubiotic microbiota influences many physiological processes in the metazoan host, including development and intestinal homeostasis. Here, we have shown that the intestinal microbiota modulates inflammatory responses caused by sex steroid deficiency, leading to trabecular bone loss. In murine models, sex steroid deficiency increased gut permeability, expanded Th17 cells, and upregulated the osteoclastogenic cytokines TNFα (TNF), RANKL, and IL-17 in the small intestine and the BM. In germ-free (GF) mice, sex steroid deficiency failed to increase osteoclastogenic cytokine production, stimulate bone resorption, and cause trabecular bone loss, demonstrating that the gut microbiota is central in sex steroid deficiency–induced trabecular bone loss. Furthermore, we demonstrated that twice-weekly treatment of sex steroid–deficient mice with the probiotics *Lactobacillus rhamnosus* GG (LGG) or the commercially available probiotic supplement VSL#3 reduces gut permeability, dampens intestinal and BM inflammation, and completely protects against bone loss. In contrast, supplementation with a nonprobiotic strain of *E. coli* or a mutant LGG was not protective. Together, these data highlight the role that the gut luminal microbiota and increased gut permeability play in triggering inflammatory pathways that are critical for inducing bone loss in sex steroid–deficient mice. Our data further suggest that probiotics that decrease gut permeability have potential as a therapeutic strategy for postmenopausal osteoporosis.

Microbiome and Bone Symposium (Monday)

Interactions of bone cells and the BM microenvironment with tumor cells determine the preference tumor cells to metastasize to and grow in bone



Excessive bone resorption: increased release of growth factors from the matrix → stimulation of tumor growth



ARTICLES Waning et al., Nat Med. 2015 Nov;21(11):1262-71

nature medicine

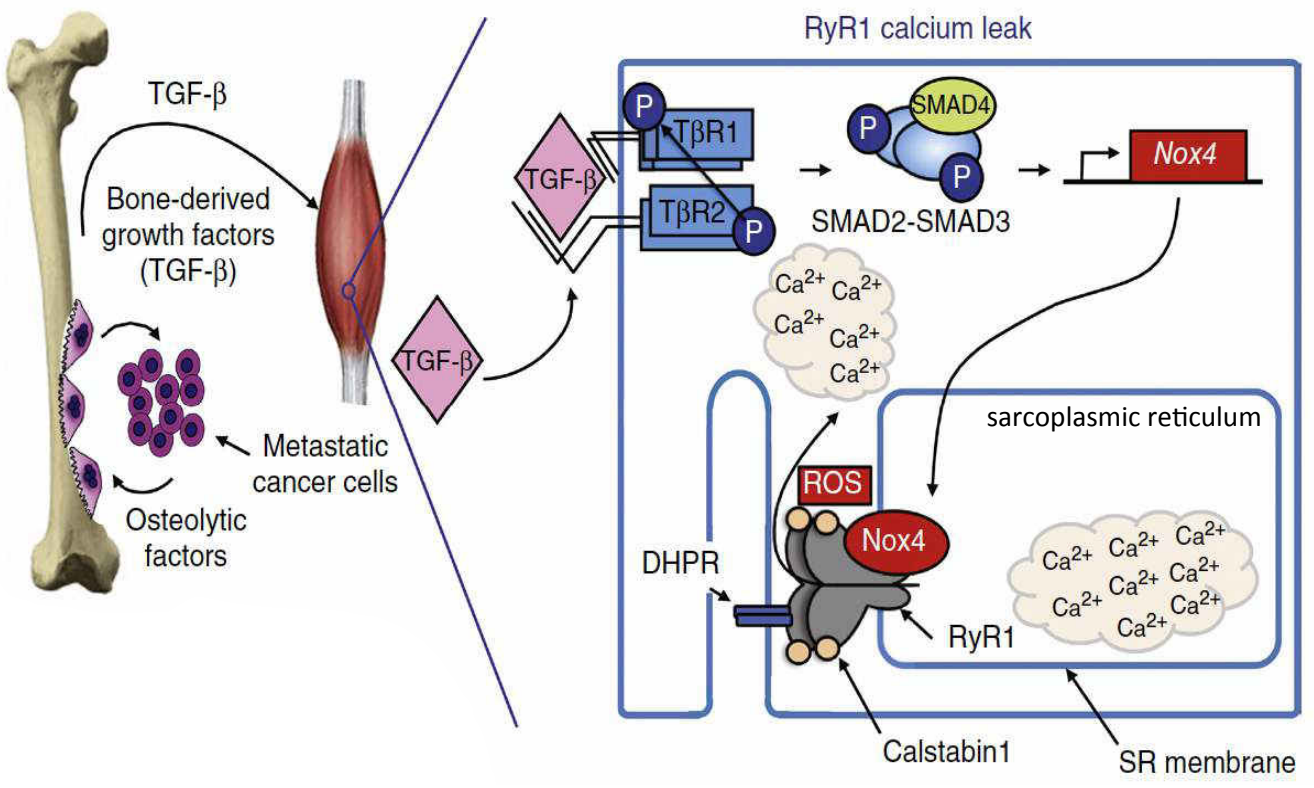
Excess TGF-β mediates muscle weakness associated with bone metastases in mice

David L Waning[1,9], Khalid S Mohammad[1,9], Steven Reiken[2], Wenjun Xie[2], Daniel C Andersson[2,8], Sutha John[1], Antonella Chiechi[1], Laura E Wright[1], Alisa Umanskaya[2], Maria Niewolna[1], Trupti Trivedi[1], Sahba Charkhzarrin[1], Pooja Khatiwada[1], Anetta Wronska[2], Ashley Haynes[2], Maria Serena Benassi[3], Frank A Witzmann[4], Gehua Zhen[5], Xiao Wang[5], Xu Cao[5], G David Roodman[6,7], Andrew R Marks[2] & Theresa A Guise[1]

Osteolytic bone metastasis in mice (from different cancers: breast, lung, prostate, multiple myeloma) are commonly associated with impaired muscle function

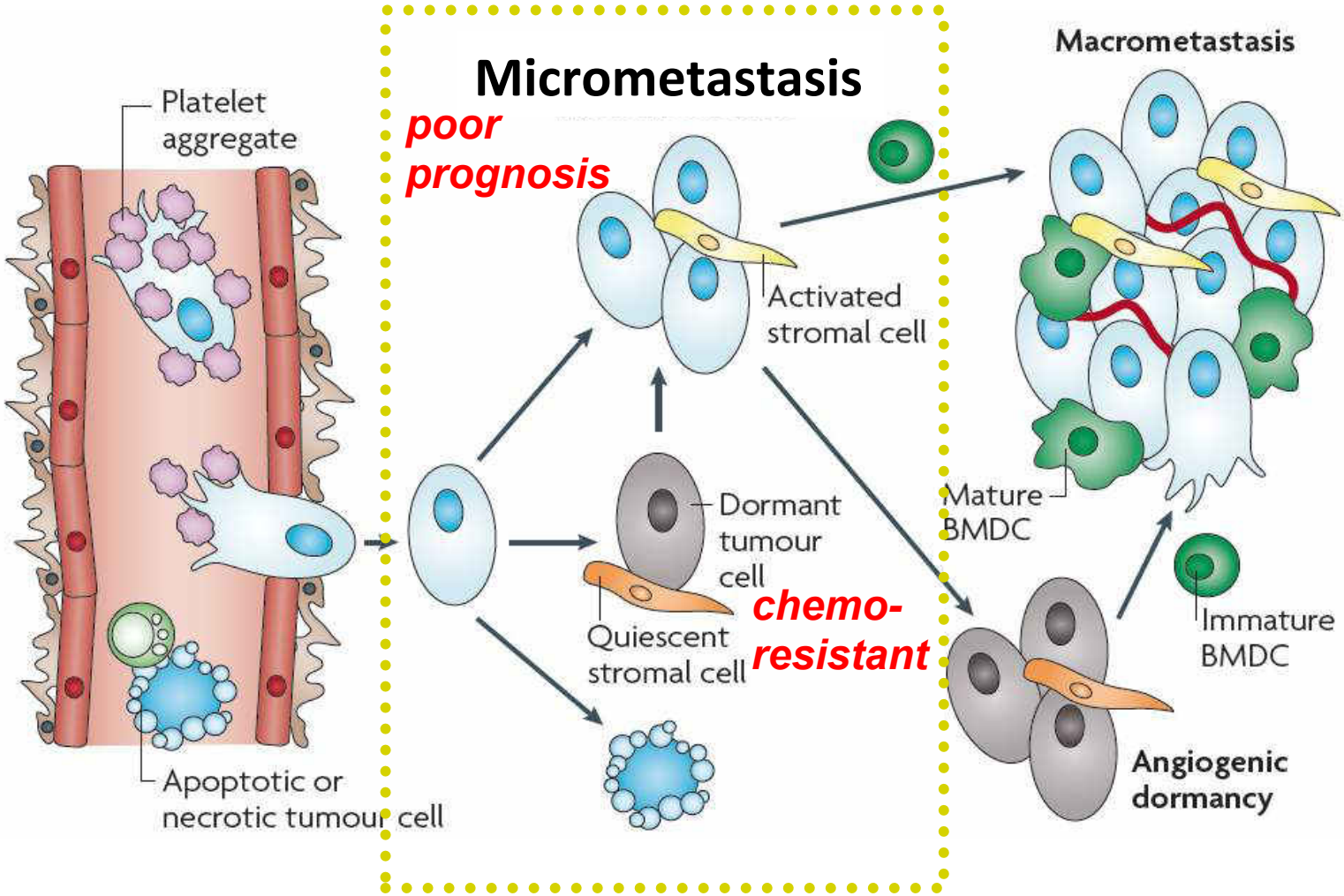
Bone-released TGF-β upregulates NADPH oxidase 4 (Nox4) in muscle

- ➔ elevated oxidization of skeletal muscle proteins, including the Ca2+ release channel RyR1
- ➔ Ca2+ leakage
- ➔ reduced intracellular signaling
- ➔ impaired muscle contraction



The pre-metastatic niche in bone

Tumor-derived factors can prime the bone environment for subsequent colonization of tumor cells, but also local stromal changes in the bone can initiate the pre-metastatic niche and drive tumor cell seeding and growth



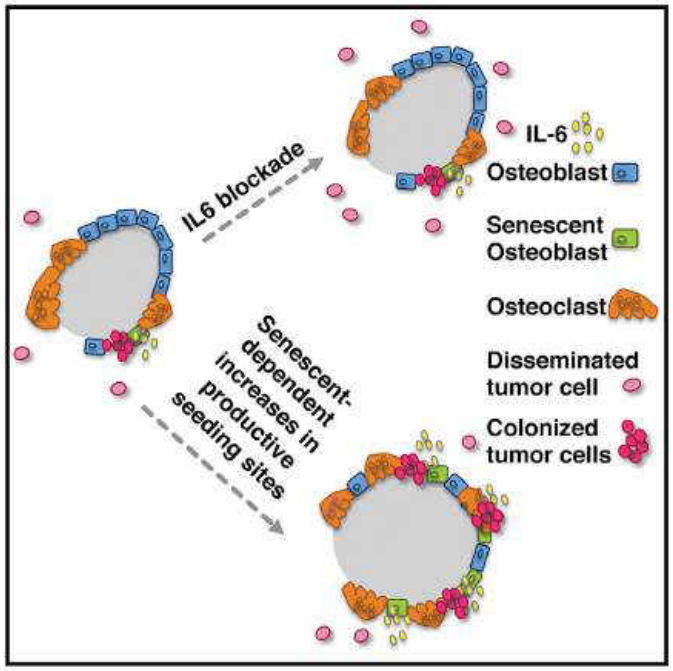
Article

Cell Reports

Stromal-Initiated Changes in the Bone Promote Metastatic Niche Development

Luo et al., Cell Rep. 2016 Jan 5;14(1):82-92

Graphical Abstract



Authors

Xianmin Luo, Yujie Fu, Andrew J. Loza, ..., Roberta Faccio, Gregory D. Longmore, Sheila A. Stewart

Correspondence

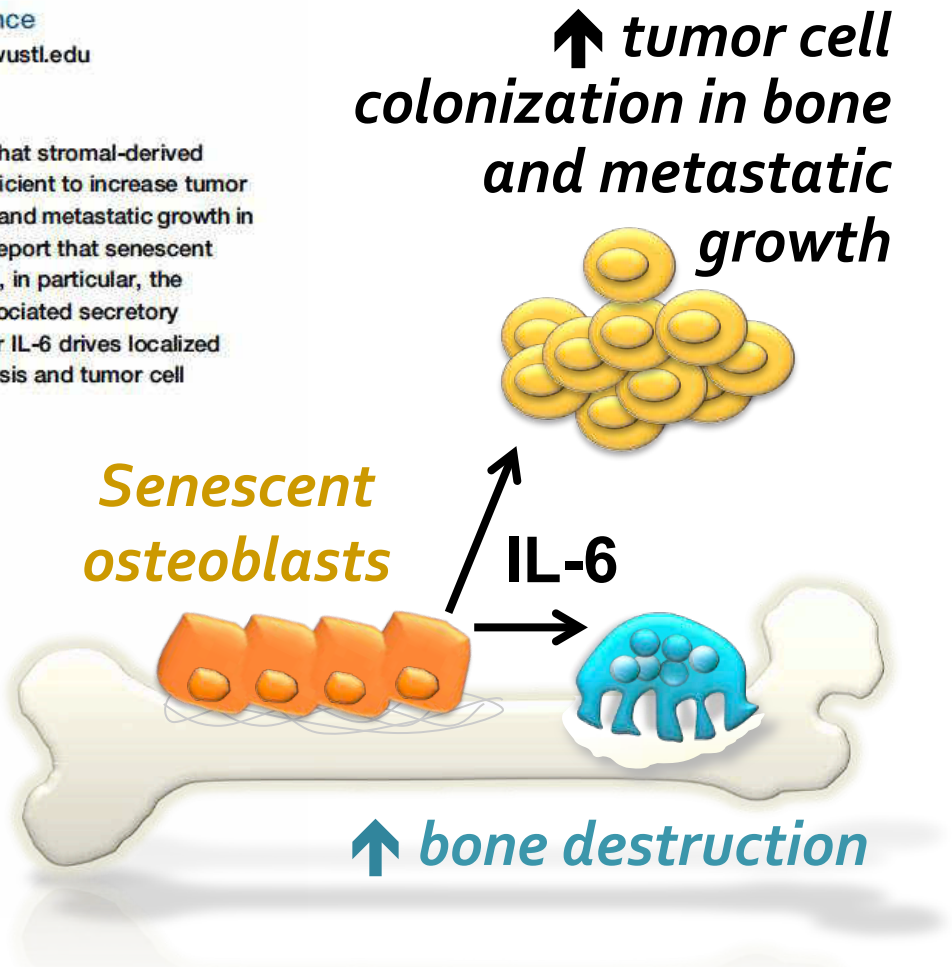
sheila.stewart@wustl.edu

In Brief

Luo et al. show that stromal-derived changes are sufficient to increase tumor cell colonization and metastatic growth in the bone. They report that senescent osteoblasts, and, in particular, the senescence-associated secretory phenotype factor IL-6 drives localized osteoclastogenesis and tumor cell growth.

Highlights

- Stromal changes in the bone drive tumor cell seeding and growth
- IL-6-expressing stromal cells are present in human bone
- Senescent osteoblasts drive increased osteoclastogenesis and tumor cell seeding
- Senescent-derived IL-6 drives localized osteoclastogenesis and tumor cell growth



Interactions between disseminating tumor cells and osteogenic cells

Cancer Cell
Article

Wang et al., Cancer Cell. 27(2):193-210, 2015.

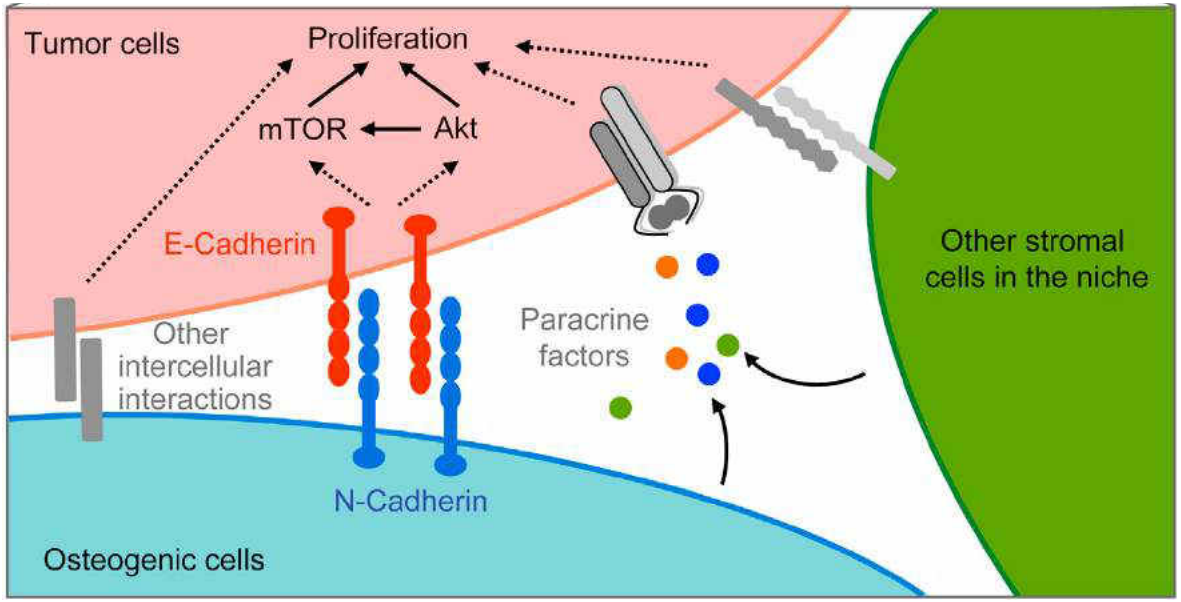
The Osteogenic Niche Promotes Early-Stage Bone Colonization of Disseminated Breast Cancer Cells

Hai Wang,[1,3,14] Cuijuan Yu,[1,3,14] Xia Gao,[1,3] Thomas Welte,[1,3] Aaron M. Muscarella,[1,6] Lin Tian,[1,2,5] Hong Zhao,[8,9] Zhen Zhao,[8,9,10] Shiyu Du,[11] Jianning Tao,[4] Brendan Lee,[4] Thomas F. Westbrook,[2,4,5] Stephen T.C. Wong,[2,3,8,9,12] Xin Jin,[13] Jeffrey M. Rosen,[2,3] C. Kent Osborne,[1,2,3] and Xiang H.-F. Zhang[1,2,3,7,*]

Heterotypic adherens junctions:

Tumor cell
E-cadherin

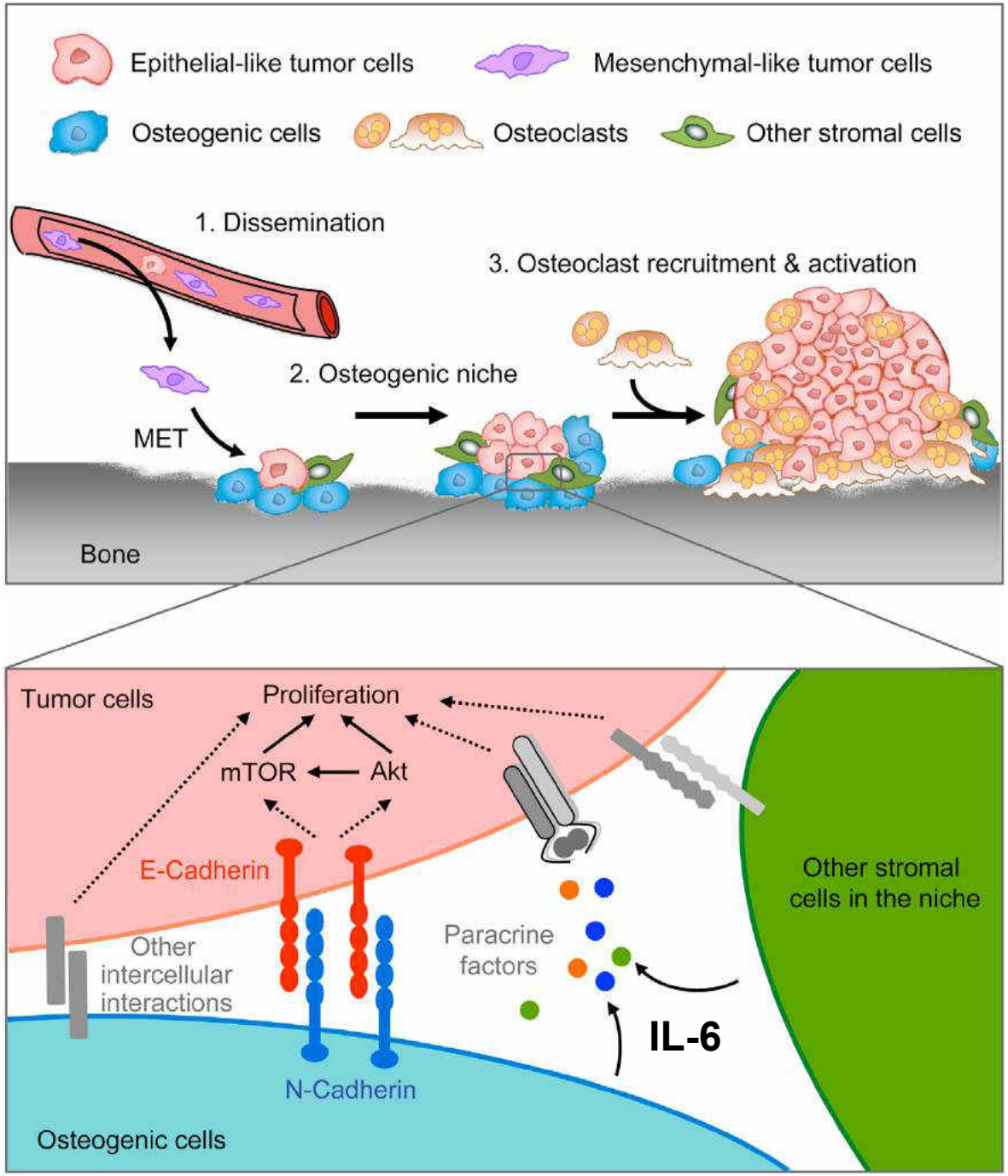
Osteogenic cell
N-cadherin



"The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase"
Cox et al., **Nature**. 2015 Jun 4;522(7554):106-10.

"ITGBL1 Is a Runx2 Transcriptional Target and Promotes Breast Cancer Bone Metastasis by Activating the TGFβ Signaling Pathway"
Li et al., **Cancer Res**. 2015 Aug 15;75(16):3302-13.

CABS Sessions at this meeting

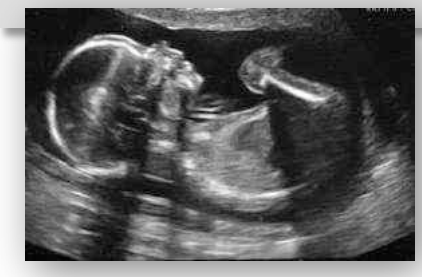


Cradle of Evil: Osteogenic Niche for Early Bone Metastasis

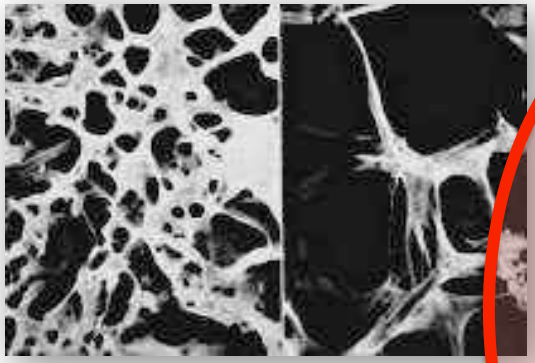
Zheng and Kang. Cancer Cell 2015

Towards applications:
In Search of the Skeletal Stem Cell

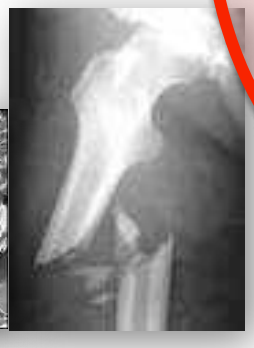
Development
Bone growth



Bone formation & Homeostasis



Fracture Repair & Tissue Engineering



MSC

Mesenchymal Stem cell

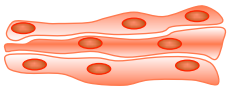
Mesenchymal Progenitor

Multipotent Stromal cell

SSC

MyoD, Myf5, myogenin

Myoblast → Myocytes



C/EBPs, PPARγ

Pre-adipocyte → Adipocytes

Runx2

Osteo-chondro-progenitor

Sox 9,5,6

Chondrocytes

Runx2, Osx, β-catenin

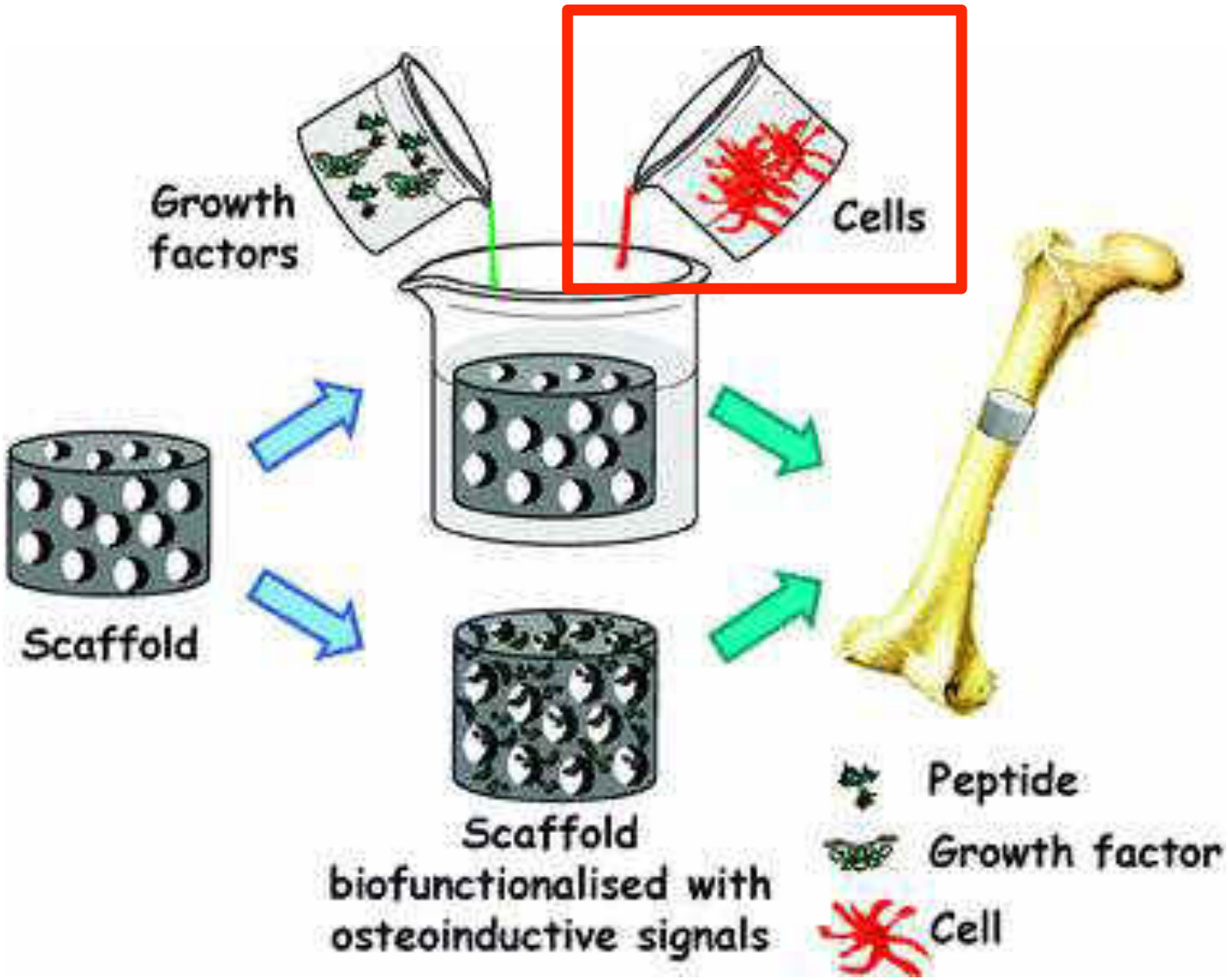
Pre-osteoblasts

Runx2, Osx

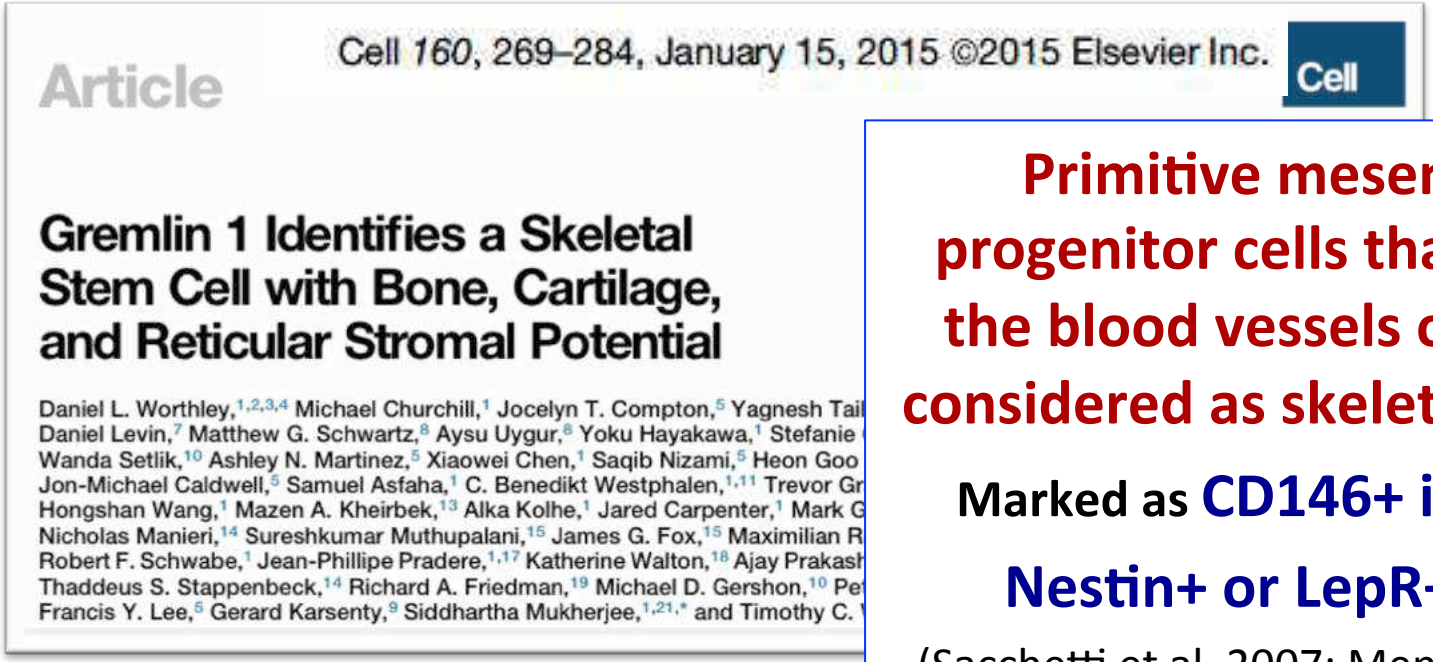
Osteoblasts

Collagen 1, ALP, Osteopontin, Osteocalcin

Fracture repair: Bone Tissue Engineering



In search of the Skeletal Stem Cell



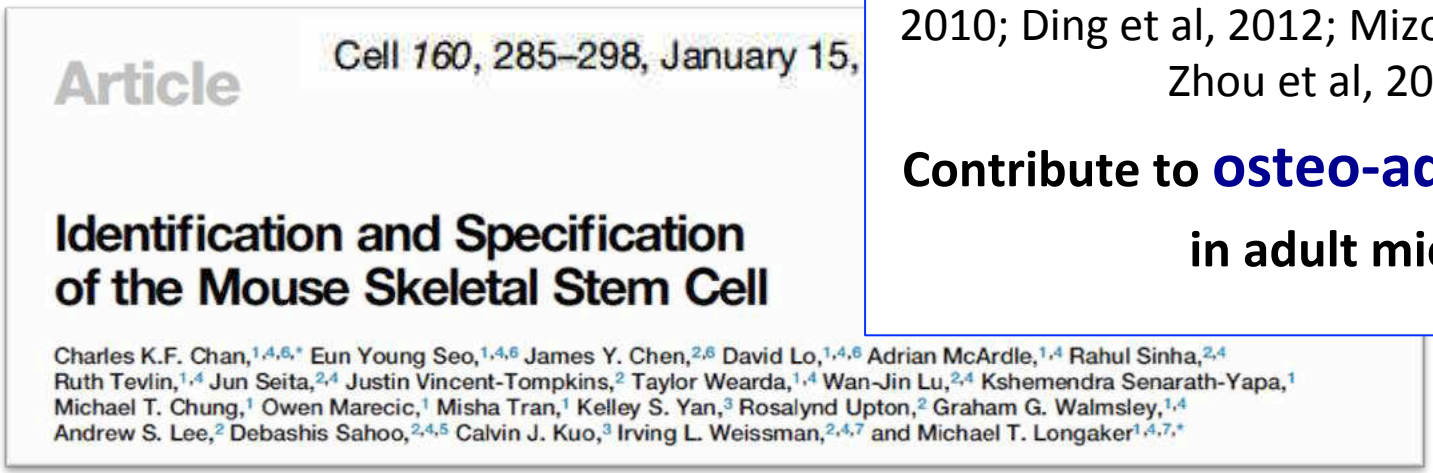
Article

Cell *160*, 269–284, January 15, 2015 ©2015 Elsevier Inc.

Cell

Gremlin 1 Identifies a Skeletal Stem Cell with Bone, Cartilage, and Reticular Stromal Potential

Daniel L. Worthley,[1,2,3,4] Michael Churchill,[1] Jocelyn T. Compton,[5] Yagnesh Tail
Daniel Levin,[7] Matthew G. Schwartz,[8] Aysu Uygur,[8] Yoku Hayakawa,[1] Stefanie
Wanda Setlik,[10] Ashley N. Martinez,[5] Xiaowei Chen,[1] Saqib Nizami,[5] Heon Goo
Jon-Michael Caldwell,[5] Samuel Asfaha,[1] C. Benedikt Westphalen,[1,11] Trevor Gr
Hongshan Wang,[1] Mazen A. Kheirbek,[13] Alka Kolhe,[1] Jared Carpenter,[1] Mark G
Nicholas Manieri,[14] Sureshkumar Muthupalani,[15] James G. Fox,[15] Maximilian R
Robert F. Schwabe,[1] Jean-Phillipe Pradere,[1,17] Katherine Walton,[18] Ajay Prakash
Thaddeus S. Stappenbeck,[14] Richard A. Friedman,[19] Michael D. Gershon,[10] Pe
Francis Y. Lee,[5] Gerard Karsenty,[9] Siddhartha Mukherjee,[1,21,*] and Timothy C.



Article

Cell *160*, 285–298, January 15,

Identification and Specification of the Mouse Skeletal Stem Cell

Charles K.F. Chan,[1,4,6,*] Eun Young Seo,[1,4,6] James Y. Chen,[2,6] David Lo,[1,4,6] Adrian McArdle,[1,4] Rahul Sinha,[2,4]
Ruth Tevlin,[1,4] Jun Seita,[2,4] Justin Vincent-Tompkins,[2] Taylor Wearda,[1,4] Wan-Jin Lu,[2,4] Kshemendra Senarath-Yapa,[1]
Michael T. Chung,[1] Owen Marecic,[1] Misha Tran,[1] Kelley S. Yan,[3] Rosalynd Upton,[2] Graham G. Walmsley,[1,4]
Andrew S. Lee,[2] Debashis Sahoo,[2,4,5] Calvin J. Kuo,[3] Irving L. Weissman,[2,4,7] and Michael T. Longaker[1,4,7,*]

Primitive mesenchymal progenitor cells that surround the blood vessels of bone are considered as skeletal stem cells

Marked as CD146+ in humans,

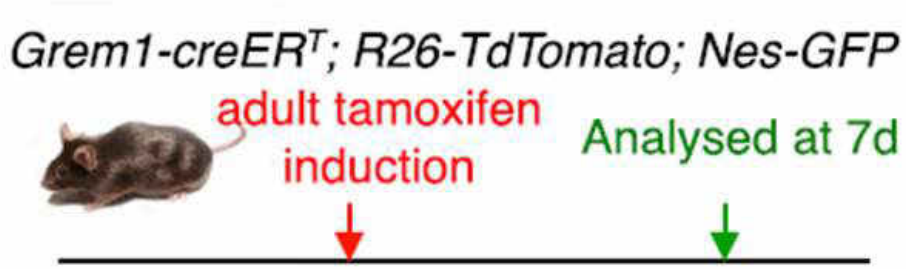
Nestin+ or LepR+ in mice

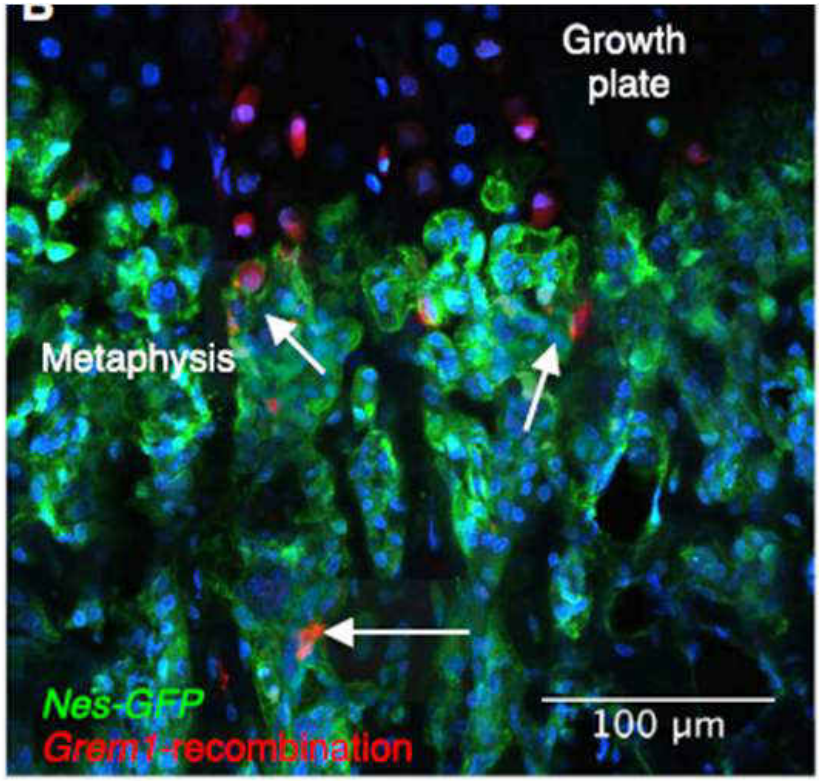
(Sacchetti et al, 2007; Mendez-Ferrer et al, 2010; Ding et al, 2012; Mizoguchi et al 2014; Zhou et al, 2014)

Contribute to osteo-adipo lineages in adult mice

In search of the Skeletal Stem Cell

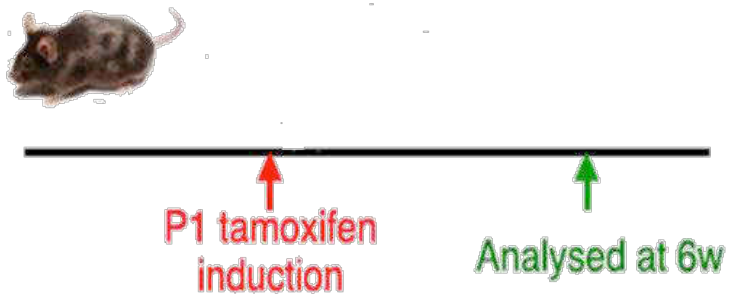
Worthley et al., Cell 160:269-284, 2015



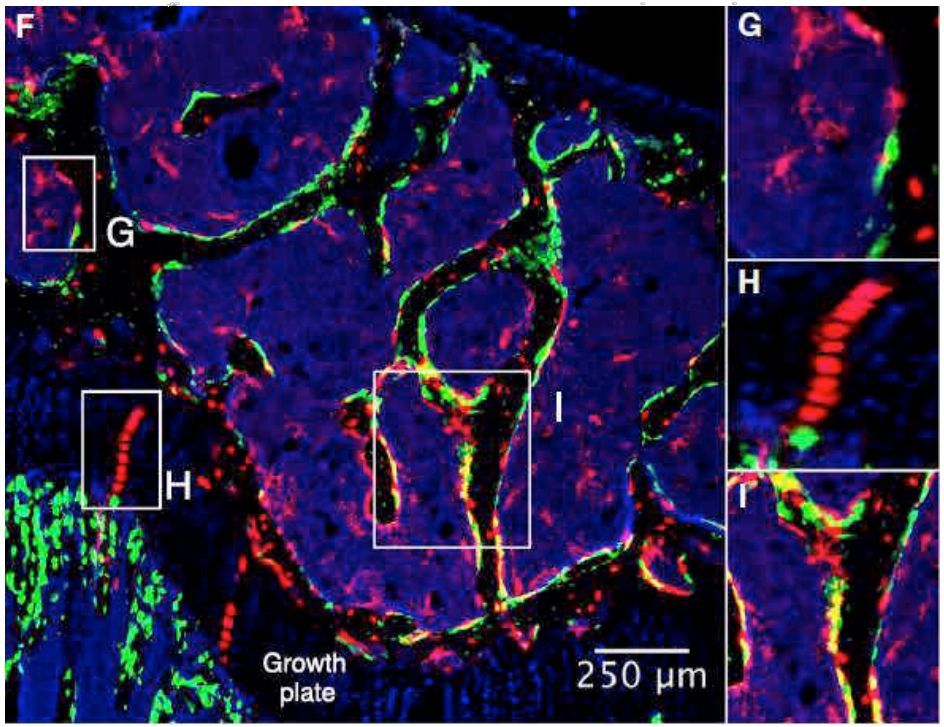


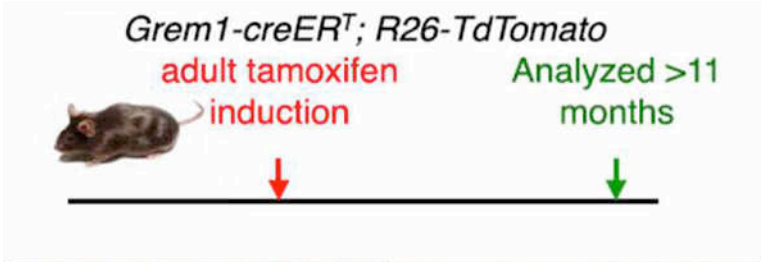
- Grem1+ cells are distinct from Nes-GFP+ cells
- 50% of Grem+ are CD105+
- <u>Single clones gave rise to osteoblasts, chondrocytes and myofibroblasts in vitro</u>

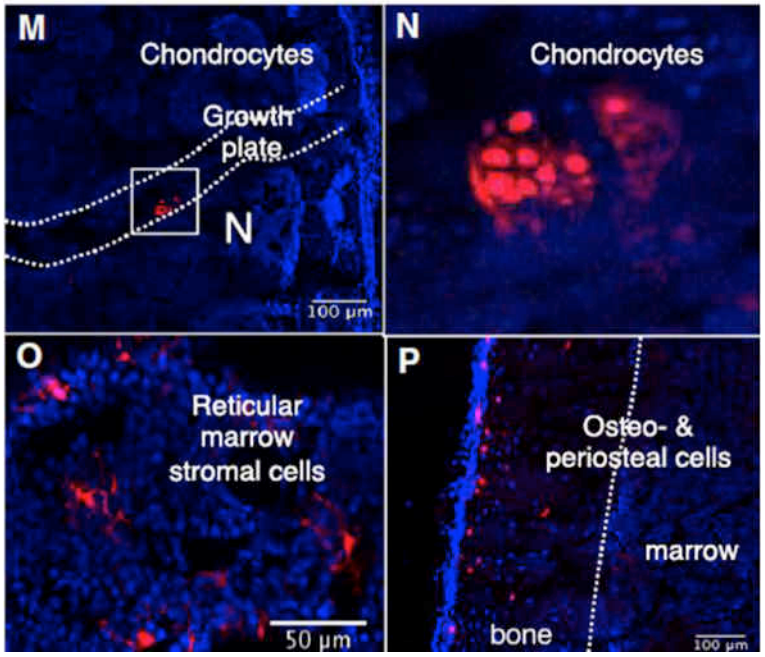
Endogenous Grem1+ cells lineage trace bone, cartilage and stroma in vivo



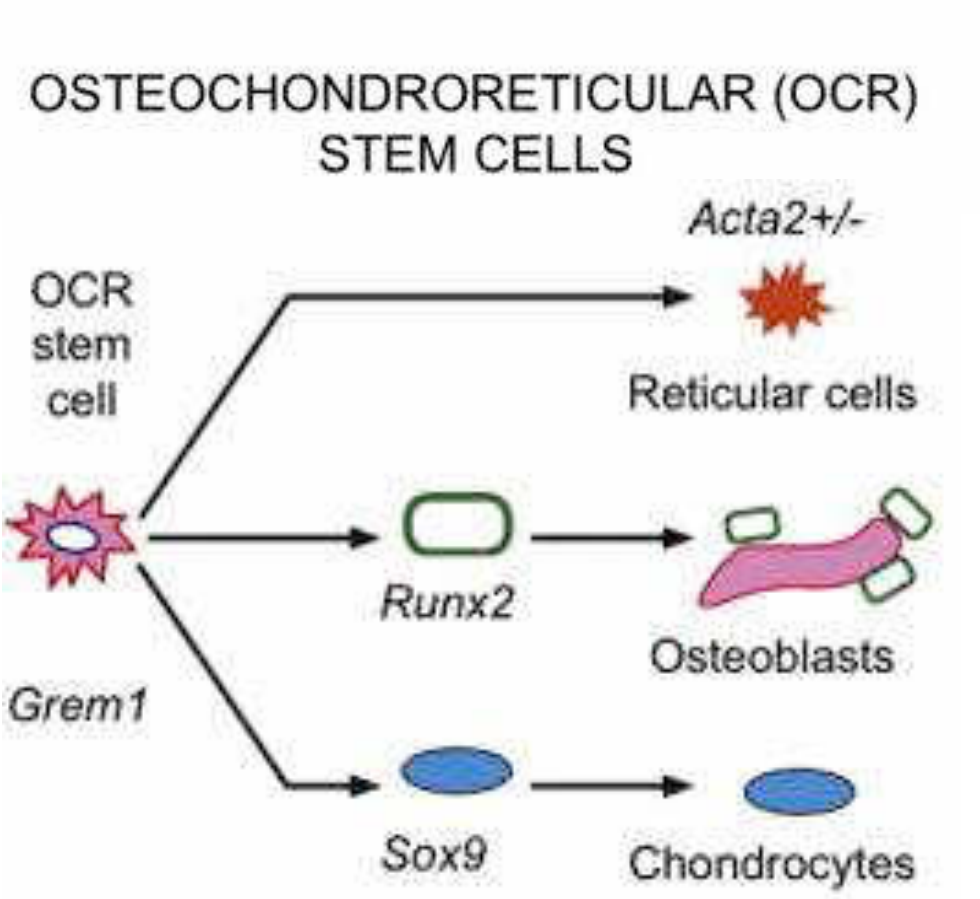
Grem1-creER^T; R26-LSL-TdTomato;2.3colGFP







Gremlin1 marks a stem cell with osteo-chondro-reticular potential (OCR cell)



- **Not peri-sinusoidal**
- **No adipogenic potential**
- **Plays a role in development, adulthood, and repair**

A complementary, non-perivascular, skeletal stem cell

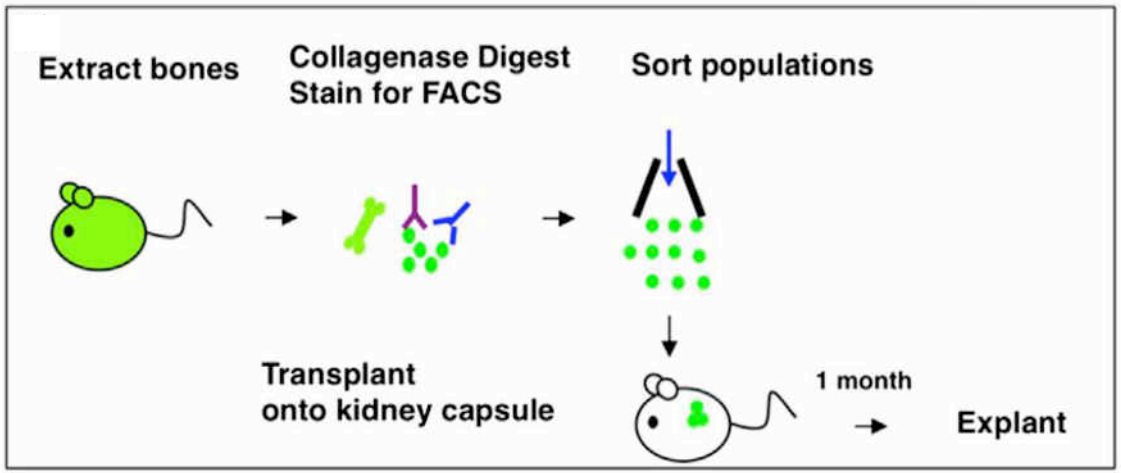
Article

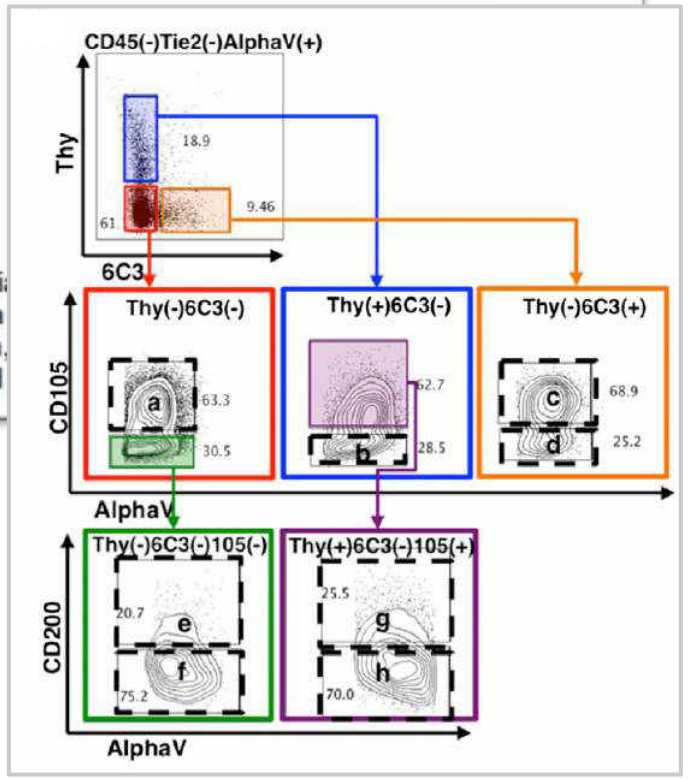
Cell *160*, 285–298, January 15, 2015 ©2015 Elsevier Inc.

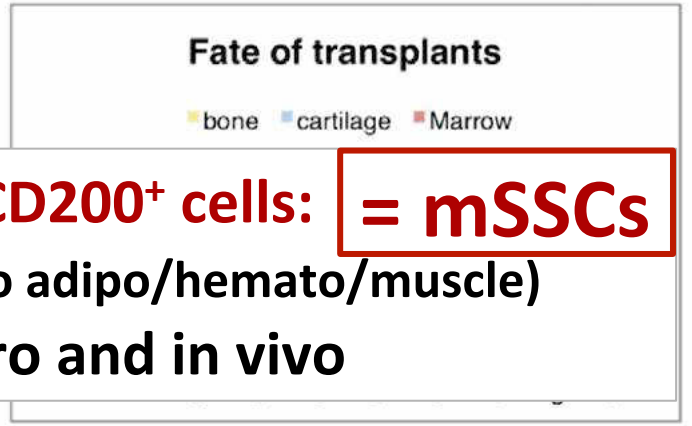
Cell

Identification and Specification of the Mouse Skeletal Stem Cell

Charles K.F. Chan,[1,4,6,*] Eun Young Seo,[1,4,6] James Y. Chen,[2,6] David Lo,[1,4,6] Adri
Ruth Tevlin,[1,4] Jun Seita,[2,4] Justin Vincent-Tompkins,[2] Taylor Wearda,[1,4] Wan-Jin
Michael T. Chung,[1] Owen Marecic,[1] Misha Tran,[1] Kelley S. Yan,[3] Rosalynd Upton,
Andrew S. Lee,[2] Debashis Sahoo,[2,4,5] Calvin J. Kuo,[3] Irving L. Weissman,[2,4,7] and



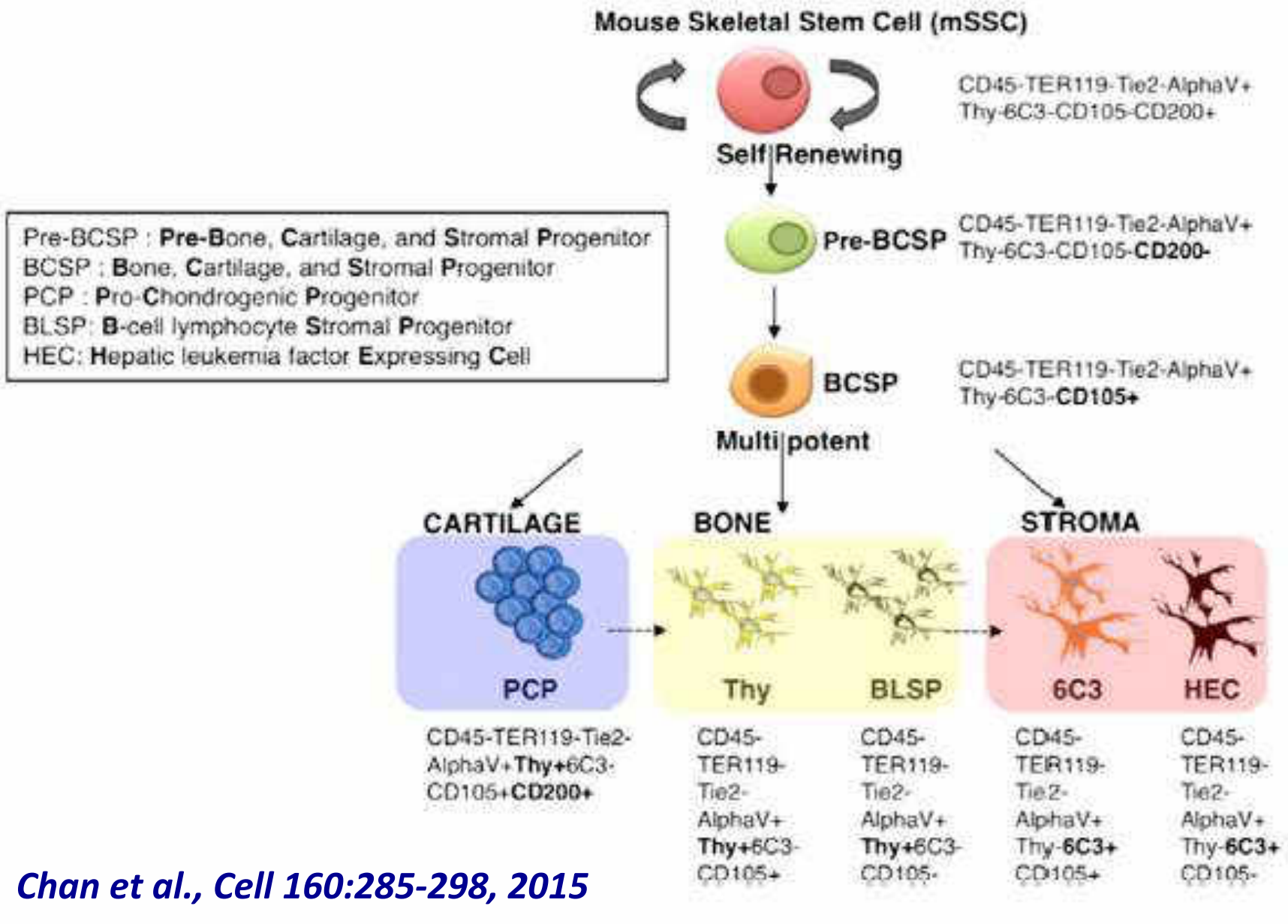




CD45$^-$ Ter119$^-$ Tie2$^-$ αv$^+$ Thy$^-$ 6C3$^-$ CD105$^-$ CD200$^+$ cells: = mSSCs

give rise to bone, cartilage, BM stroma (no adipo/hemato/muscle) and to all the other subpopulations in vitro and in vivo

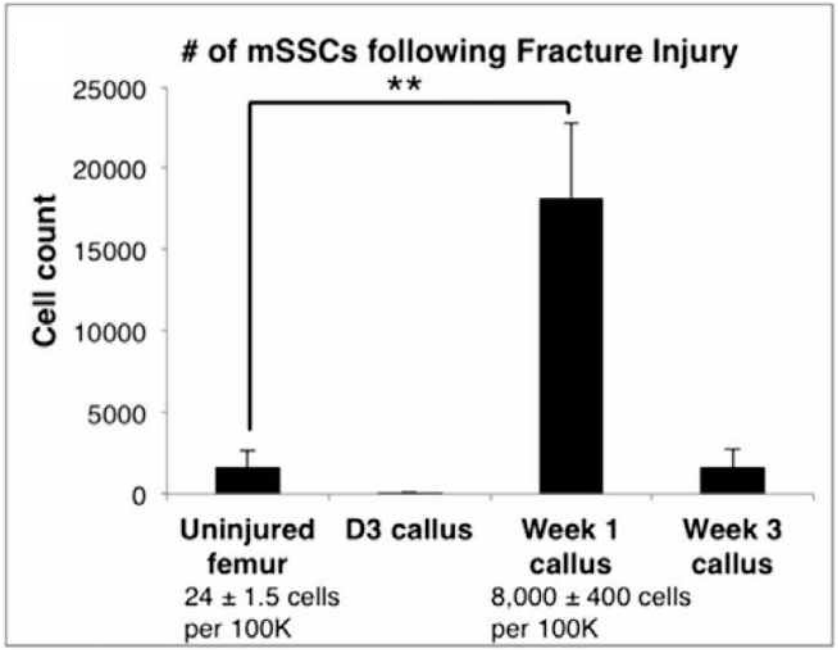
Hierarchy chart of differentiation along different lineages



Chan et al., Cell 160:285-298, 2015

Translational impact of the Skeletal Stem Cell: Fracture healing

- **mSSCs are enriched in fracture calluses and demonstrate enhanced osteogenic capacity**



- "Identification and characterization of an injury-induced skeletal progenitor"
  Marecic O, Tevlin R, McArdle A, Seo EY, Wearda T, Duldulao C, Walmsley GG, Nguyen A, Weissman IL, Chan CK, Longaker MT.
  PNAS 112:9920-5. **Aug 2015**

- "Skeletal Stem Cell Niche Aberrancies Underlie Impaired Fracture Healing in a Mouse Model of Type 2 Diabetes"
  Tevlin R, Young Seo E, Marecic O, Wearda T, Mc Ardle A, Januszyk M, Gulati G, Maan Z, Hu MS, Walmsley GG, Gurtner GC, Chan CK, Weissman IL, Longaker MT.
  Plast Reconstr Surg. 136(4 Suppl):73. **Oct 2015**

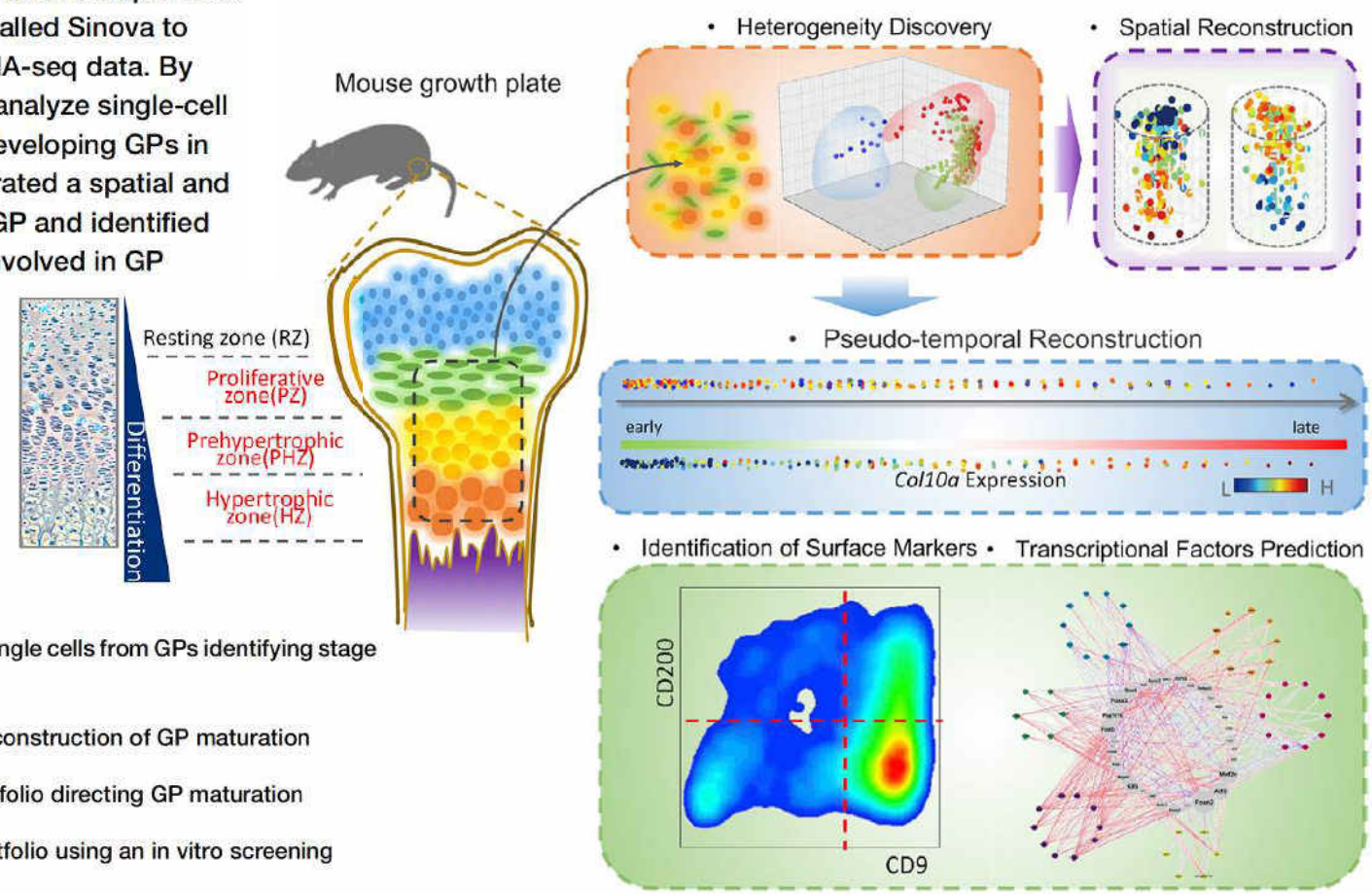
Stem Cells Workshop (Monday)

Use of new technologies

Systematic Reconstruction of Molecular Cascades Regulating GP Development Using Single-Cell RNA-Seq

Li et al., Cell Reports. pii: S2211-1247(16)30470-3. May 2016

Li et al. have developed an unsupervised clustering approach called Sinova to analyze single-cell RNA-seq data. By using this pipeline to analyze single-cell RNA-seq data from developing GPs in mice, they have generated a spatial and temporal map of the GP and identified molecular networks involved in GP development.

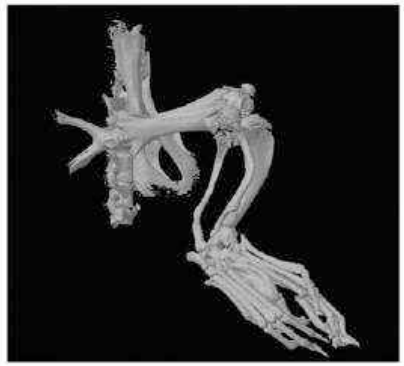


Highlights

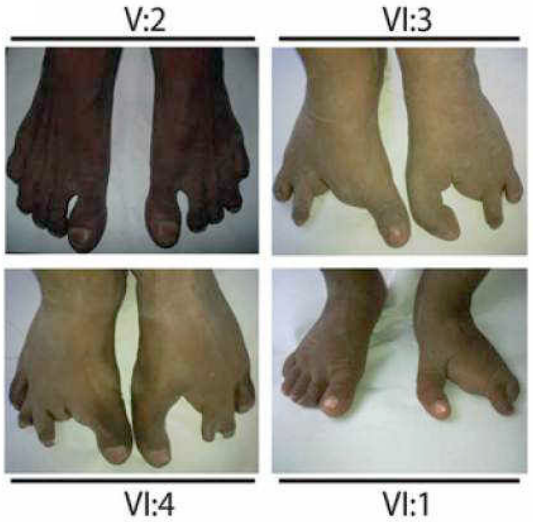
- Unbiased clustering of single cells from GPs identifying stage transitions
- Temporal and spatial reconstruction of GP maturation
- Prediction of the TF portfolio directing GP maturation
- Recapitulation of TF portfolio using an in vitro screening system

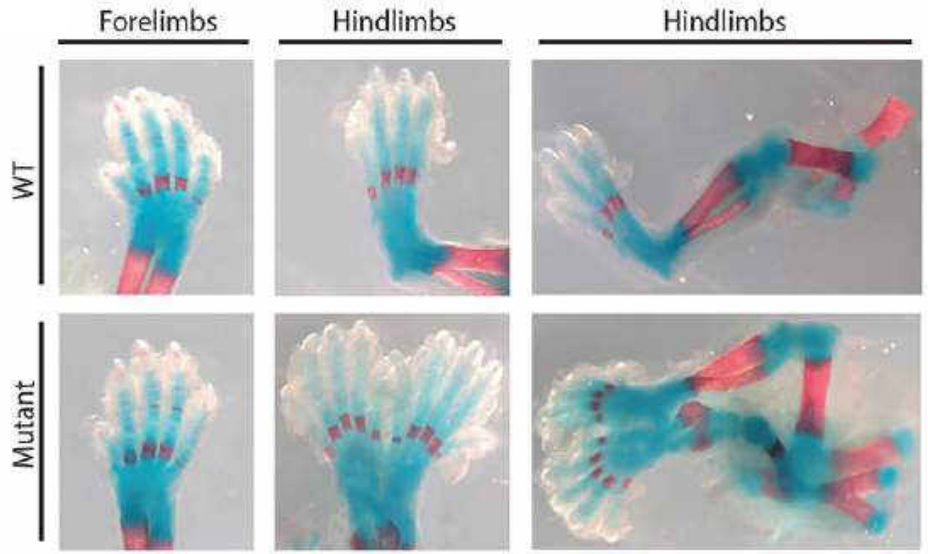
Symposium 2 (today 14:30)

Use of new technologies



Exome sequencing and CRISPR/Cas genome editing identify mutations of *ZAK* as a cause of limb defects in humans and mice



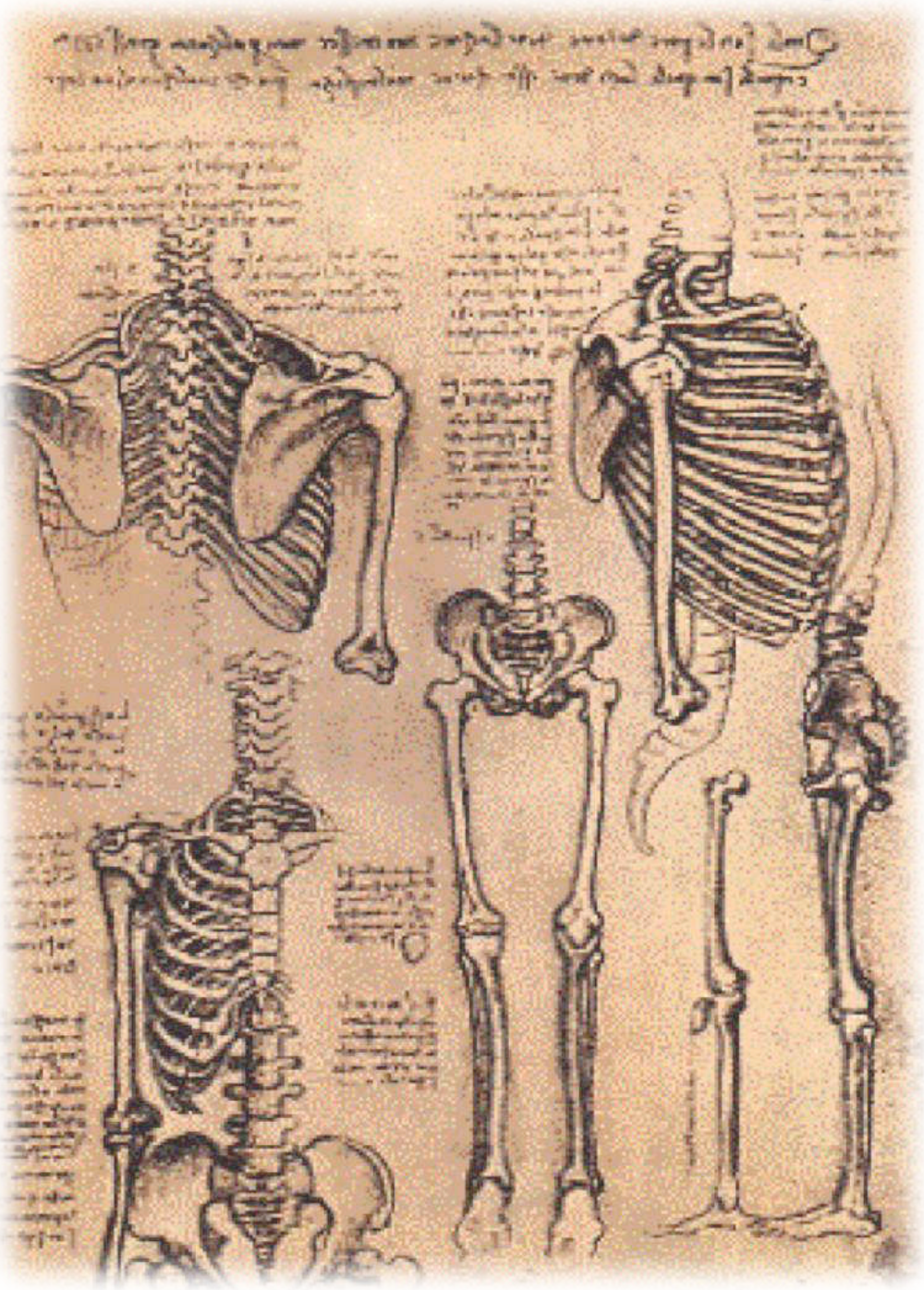


The CRISPR/Cas technology enables targeted genome editing and the rapid generation of transgenic animal models for the study of human genetic disorders. Here we describe an autosomal recessive human disease in two unrelated families characterized by a split-foot defect, nail abnormalities of the hands, and hearing loss, due to mutations disrupting the SAM domain of the protein kinase ZAK. ZAK is a member of the MAPKKK family with no known role in limb development. We show that *Zak* is expressed in the developing limbs and that a CRISPR/Cas-mediated knockout of the two *Zak* isoforms is embryonically lethal in mice. In contrast, a deletion of the SAM domain induces a complex hindlimb defect associated with down-regulation of *Trp63*, a known split-hand/split-foot malformation disease gene. Our results identify ZAK as a key player in mammalian limb patterning and demonstrate the rapid utility of CRISPR/Cas genome editing to assign causality to human mutations in the mouse in <10 wk.

Spielmann et al., Genome Res. 26:183-191, Feb 2016

LGR4 is a receptor for RANKL and negatively regulates osteoclast differentiation and bone resorption.
Luo J, Yang Z, Ma Y, Yue Z, Lin H, Qu G, Huang J, Dai W, Li C, Zheng C, Xu L, Chen H, Wang J, Li D, Siwko S, Penninger JM, Ning G, Xiao J, Liu M. **Nat Med.** 2016 May;22(5):539-46.
Identification of a Prg4-expressing articular cartilage progenitor cell population in mice.
Kozhemyakina E, Zhang M, Ionescu A, Ayturk UM, Ono N, Kobayashi A, Kronenberg H, Warman ML, Lassar AB. **Arthritis Rheumatol**. 2015
Proteomic signatures of extracellular vesicles secreted by nonmineralizing and mineralizing human osteoblasts and stimulation of tumor cell growth.
Morhayim J, van de Peppel J, Demmers JA, Kocer G, Nigg AL, van Driel M, Chiba H, van Leeuwen JP. **FASEB J.** 2015 Jan;29(1):274-85.
Anabolic and Antiresorptive Modulation of Bone Homeostasis by the Epigenetic Modulator Sulforaphane, a Naturally Occurring Isothiocyanate.
Thaler R, Maurizi A, Roschger P, Sturmlechner I, Khani F, Spitzer S, Rumpler M, Zwerina J, Karlic H, Dudakovic A, Klaushofer K, Teti A, Rucci N, Varga F, van Wijnen AJ**. J Biol Chem**. 2016 Mar 25;291(13):6754-71.
Effective Small Interfering RNA Therapy to Treat CLCN7-dependent Autosomal Dominant Osteopetrosis Type 2.
Capulli M, Maurizi A, Ventura L, Rucci N, Teti A. **Mol Ther Nucleic Acids**. 2015 Sep 1;4:e248.
Missense Mutations in LRP5 Associated with High Bone Mass Protect the Mouse Skeleton from Disuse- and Ovariectomy-Induced Osteopenia.
Niziolek PJ, Bullock W, Warman ML, Robling AG. **PLoS One**. 2015 Nov 10;10(11):e0140775.
FGF signaling in the osteoprogenitor lineage non-autonomously regulates postnatal chondrocyte proliferation and skeletal growth.
Karuppaiah K, Yu K, Lim J, Chen J, Smith C, Long F, Ornitz DM. **Development**. 2016 Apr 6. pii: dev.131722
High Bone Mass-Causing Mutant LRP5 Receptors Are Resistant to Endogenous Inhibitors In Vivo.
Niziolek PJ, MacDonald BT, Kedlaya R, Zhang M, Bellido T, He X, Warman ML, Robling AG.
J Bone Miner Res. 2015 Oct;30(10):1822-30.
Diet-induced obesity promotes a myeloma-like condition in vivo.
Lwin ST, Olechnowicz SW, Fowler JA, Edwards CM. **Leukemia**. 2015 Feb;29(2):507-10.
Lifelong challenge of calcium homeostasis in male mice lacking TRPV5 leads to changes in bone and calcium metabolism.
van der Eerden BC, Koek WN, Roschger P, Zillikens MC, Waarsing JH, van der Kemp A, Schreuders-Koedam M, Fratzl-Zelman N, Leenen PJ, Hoenderop JG, Klaushofer K, Bindels RJ, van Leeuwen JP. **Oncotarget**. 2016 Apr 18.


What's NEW in
Basic bone research
Physiology
Osteoblasts
Mouse models
Skeletal Stem Cells
Osteoprogenitors
Signaling
Development
Cartilage
Remodeling
Fracture healing
Skeletal vascularization
Innovation
Cell Biology
Molecular Mechanisms
Osteoclasts



Basic Bone Research: A Year in review

2016 ECTS meeting
Rome

Christa Maes