Osteoporosis therapy using AAV-mediated gene transfer

Jae-Hyuck Shim, PhD

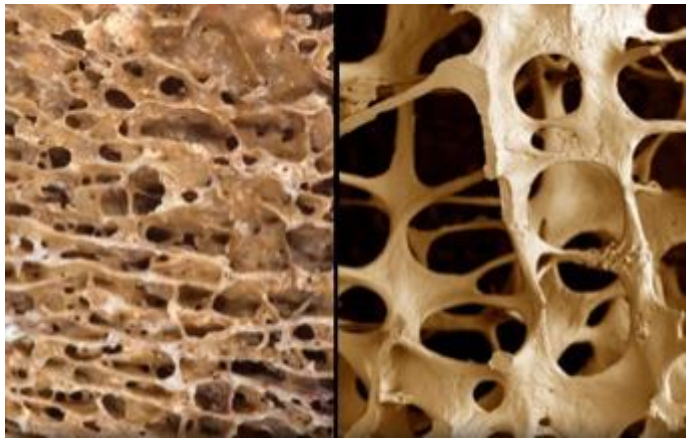
Division of Rheumatology/Medicine

University of Massachusetts Medical School

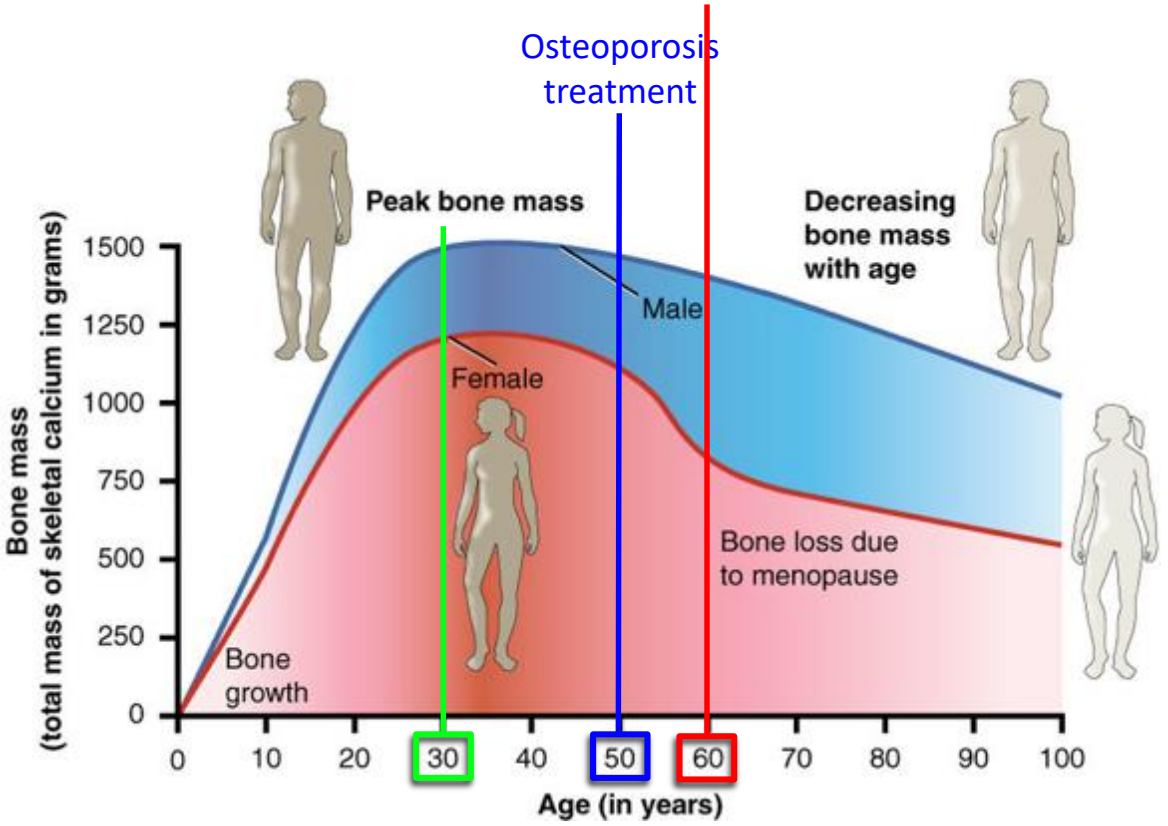
2019 AAV Gene Therapy Seminar (12/03/2019)

Osteoporosis causes severe bone loss and deterioration of bone microstructure

Normal Osteoporosis



Life span extended



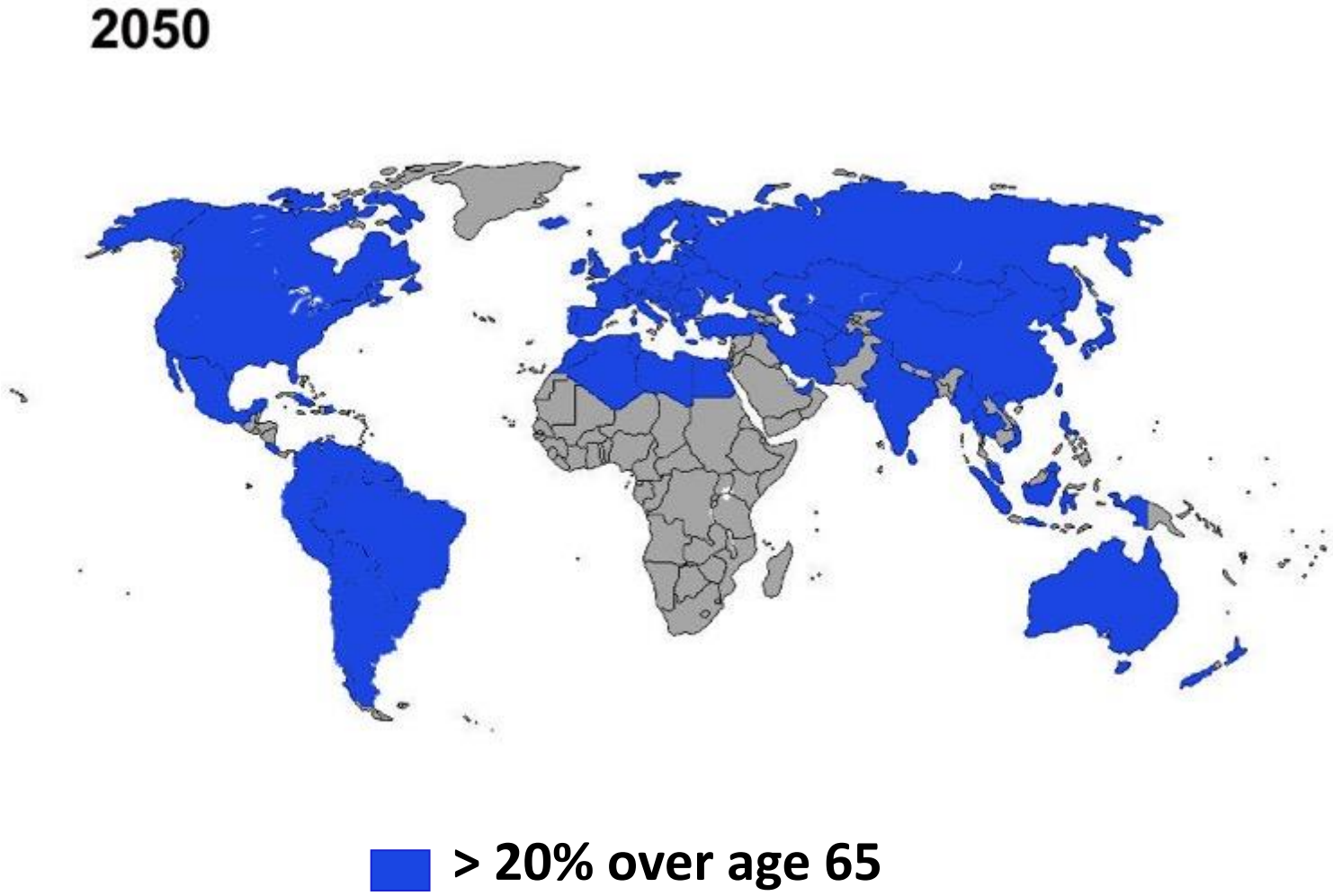
Osteoporosis is a major health issue

1. 200 million people worldwide, >40 million people in the US suffer osteoporosis.
   - 1 in 3 women and 1 in 5 men will suffer an osteoporosis-related fracture during their lifetime.
   -Afflicts 55% of Americans over the age of 50.

2. 24% of hip/spinal fracture patients over the age of 50 die in the year following their fracture due to clinical complications.

3. Limited therapeutic options to treat osteoporosis-related fracture.

Osteoporosis increases as the world population ages



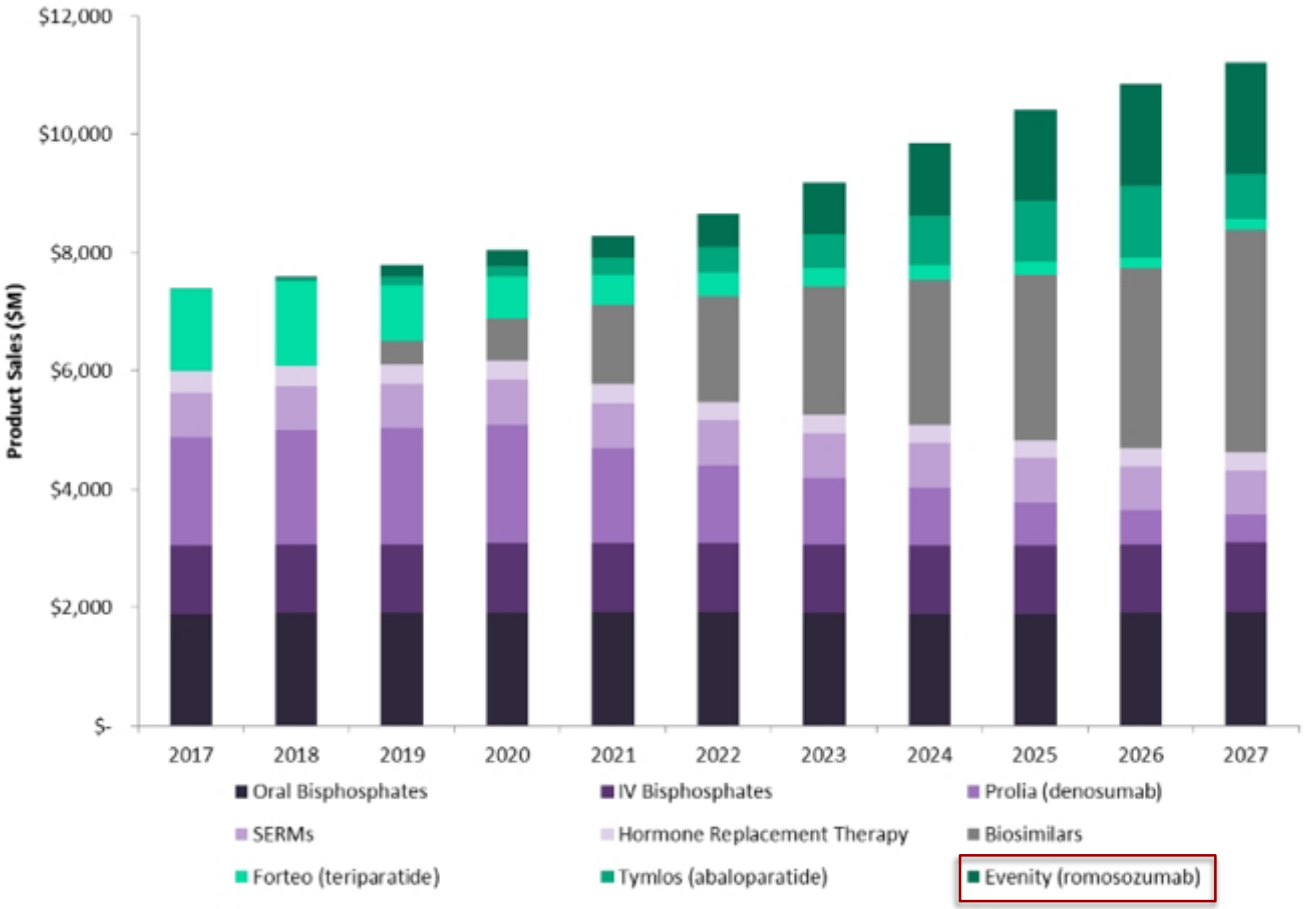
Global osteoporosis drug market

U.S. market share of prescription osteoporosis treatments

Drug (Company)	2009 U.S. market share
Actonel (Sanofi Aventis and Warner Chilcott)	20.7%
Zometa (Novartis)	16.8
Boniva (Roche)	16.6
Evista (Eli Lilly)	15.5
Forteo (Eli Lilly)	12.0
Reclast (Novartis)	7.4
Others	11.0

Total U.S. sales: $4.4 billion

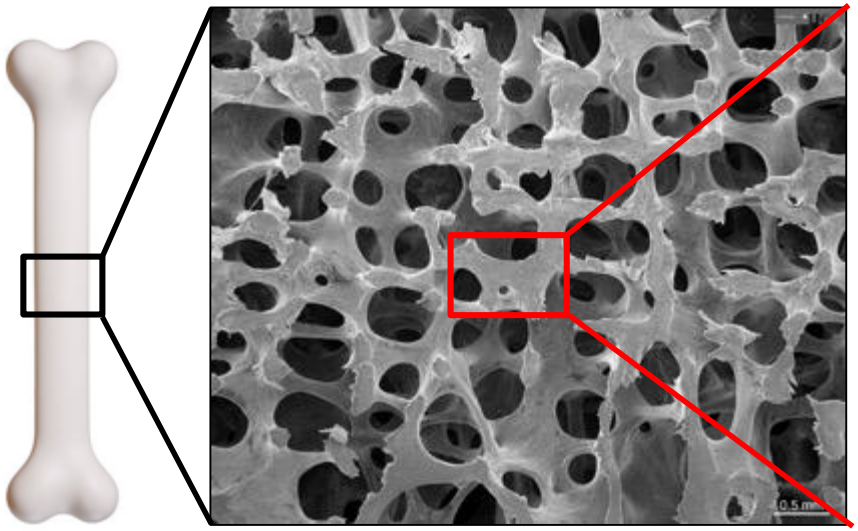
Source: IMS Health

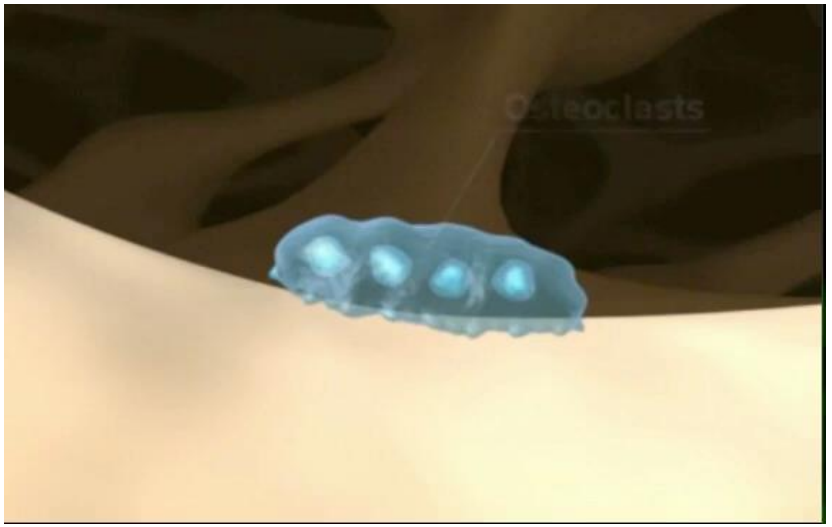


Bone is a dynamic tissue

Osteoclast
(Bone destruction)

Osteoblast
(Bone formation)

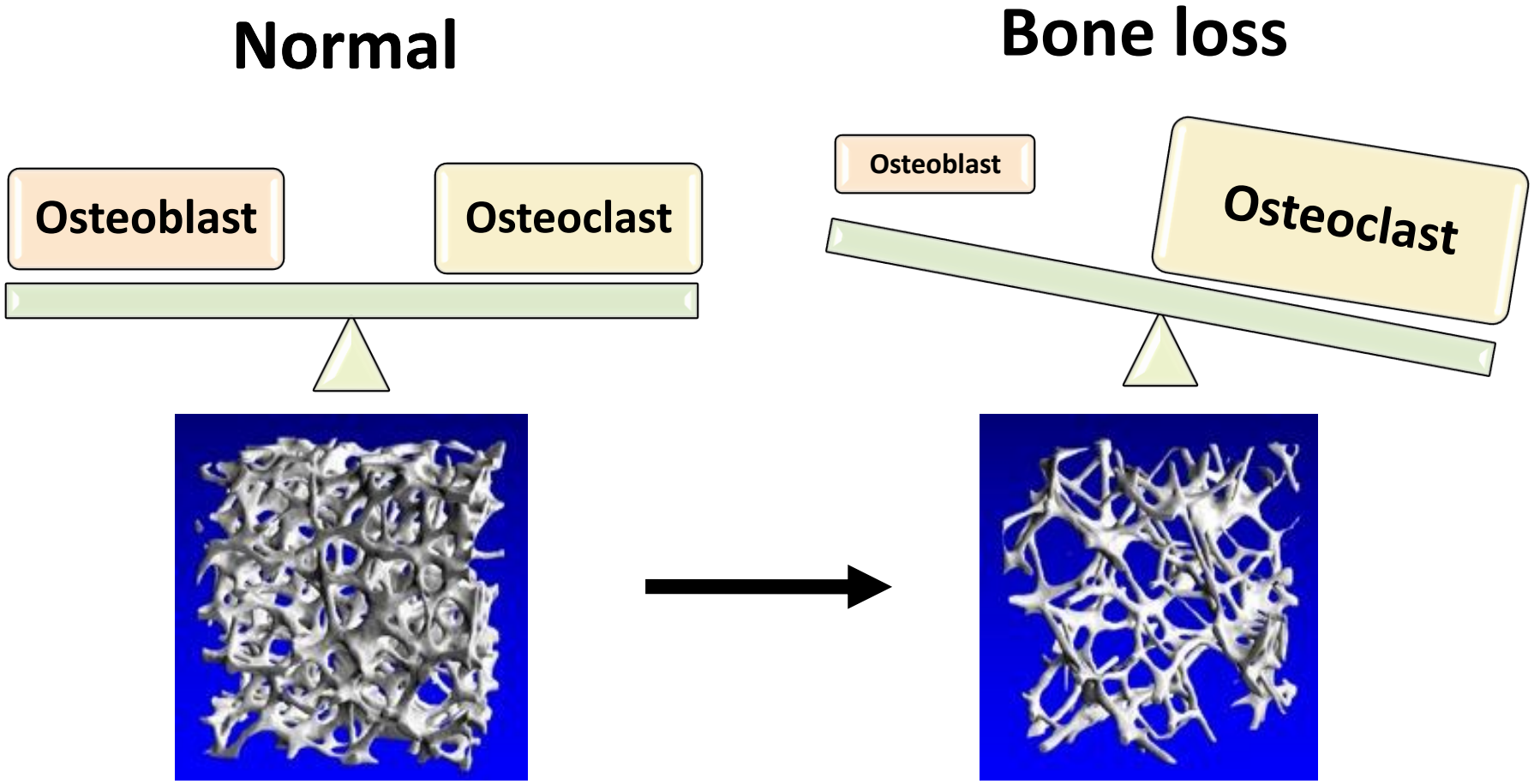




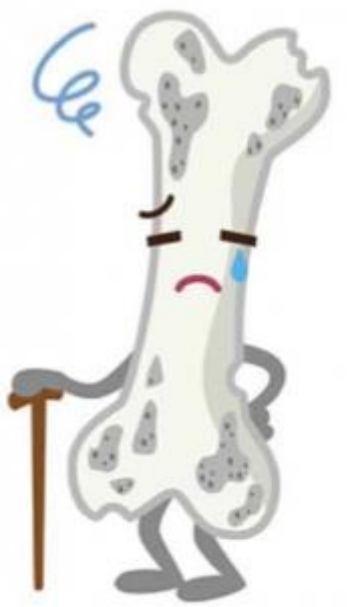
3 months/cycle

Whole body regeneration/10 years

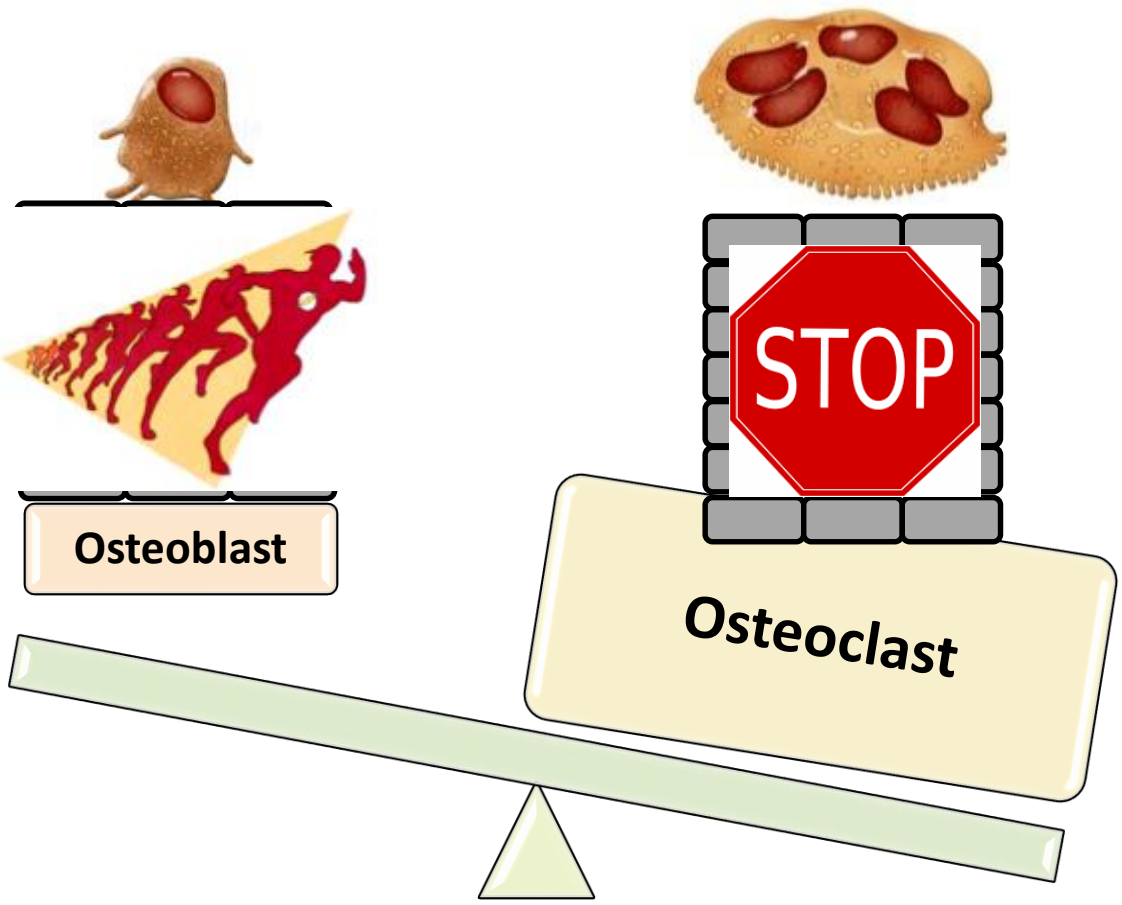
Pathogenesis of osteoporosis



How to treat bone loss in human?



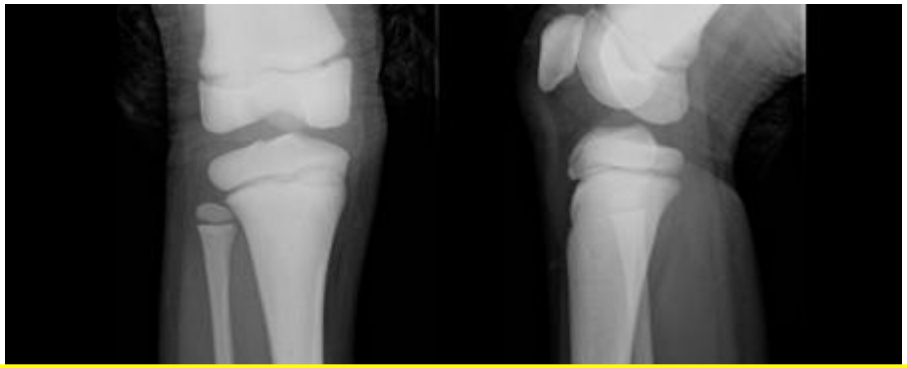
Osteoporotic bone

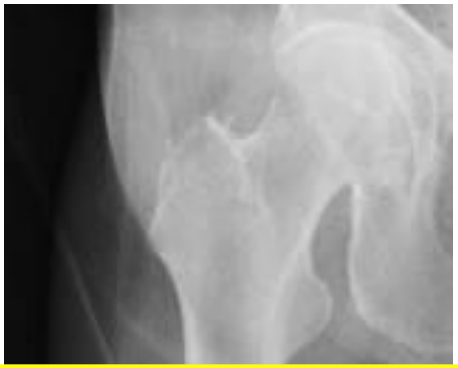


Osteonecrosis of jaws

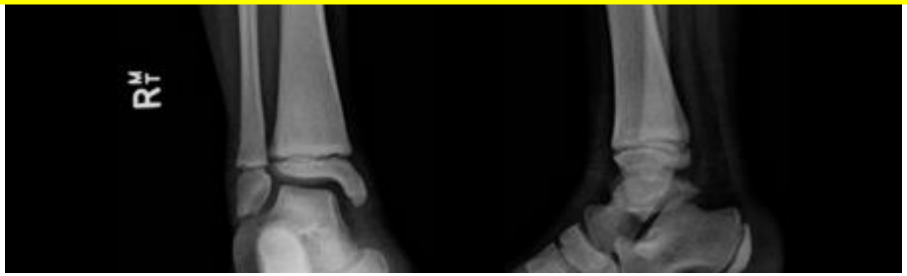
Development of new osteoporosis therapeutics with limiting adverse effects is unmet need.

Bone remodeling is required for microfracture repair



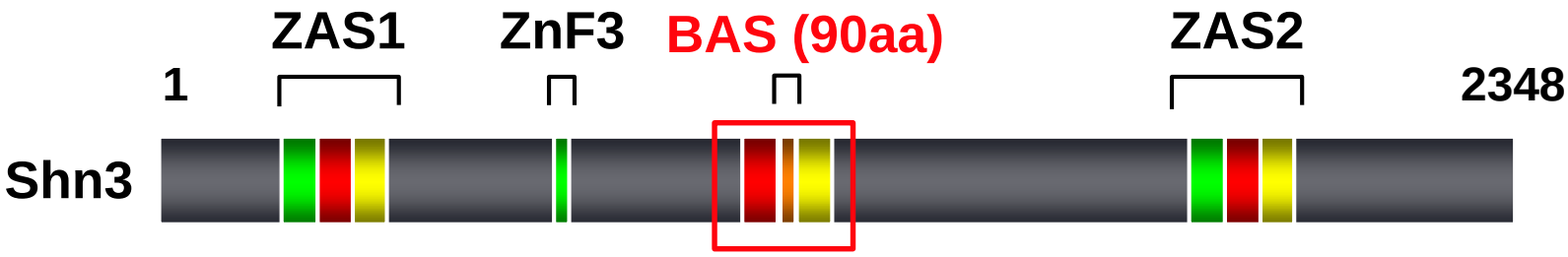


Promoting osteoblast activity is considered as a better approach to treat osteoporosis than blocking osteoclast activity



Loss of function mutations in the *Cathepsin K* gene causes Pycnodysostosis in human

Schnurri-3 (KRC, ZAS3, HIVEP3)



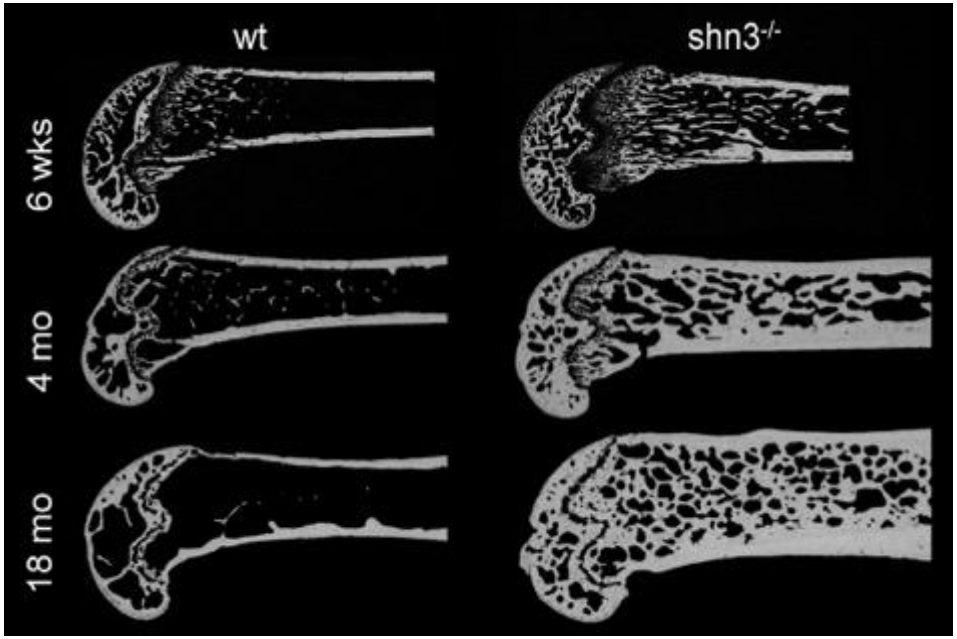
ERK binding site (PPKKKRLRL)

1. Shn3 was originally identified Th2-specific transcription factor by Laurie's group (Yeast one-hybrid screening for GATA3-binding)

2. Shn3 inhibits TRAF2-mediated NF-kB activation in TNF signaling pathway (*Oukka et al., Mol Cell, 2002*)

3. Shn3 plays a role in regulation of IL-2 induction in T cells by interacting with c-Jun (*Oukka et al., J Exp Med, 2004*)

Loss of SHN3 increases bone mass in mice



<Jones and Wein et al., Science, 2006>

Lamellar bone

No ectopic mineralization

No osteosarcomas

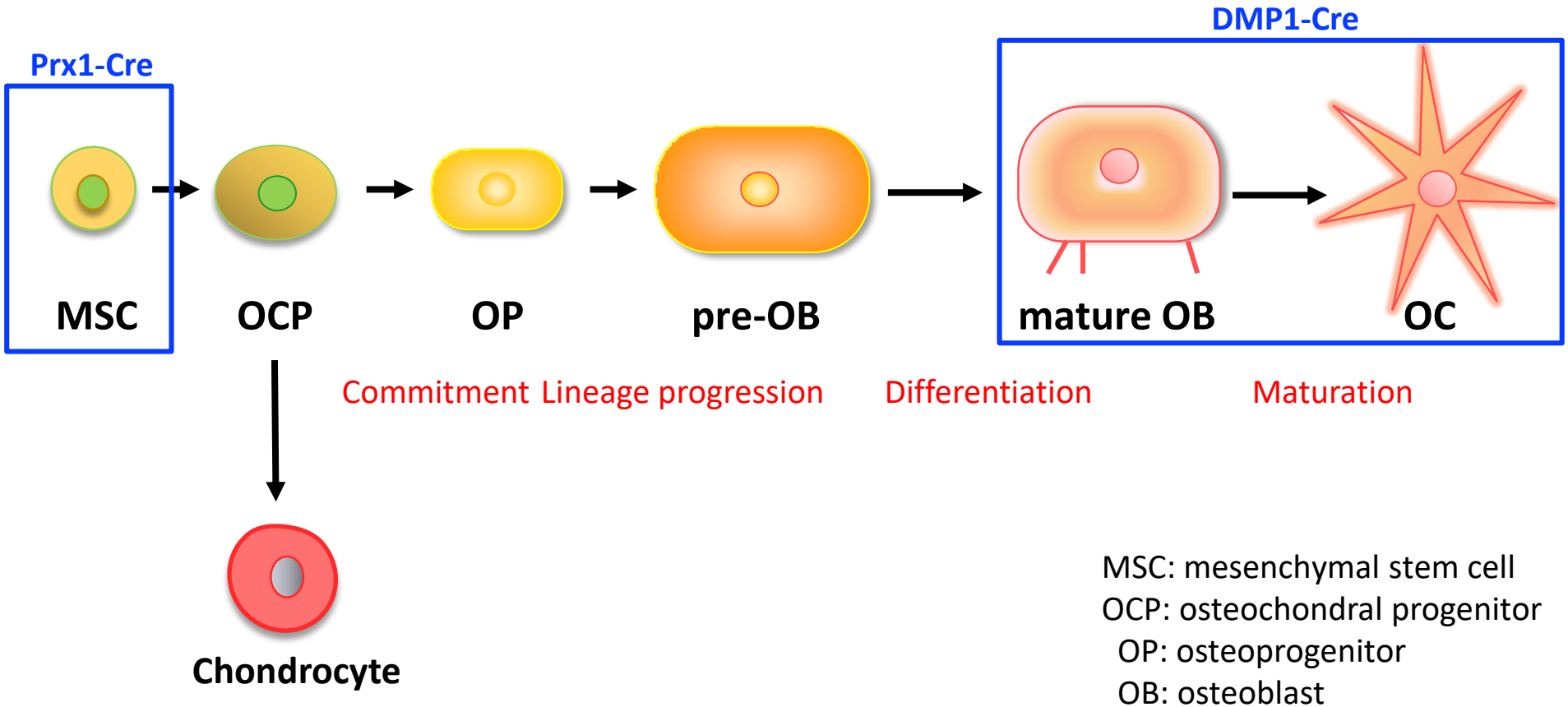
Normal joints

No metabolic abnormalities

Increased bone formation rate

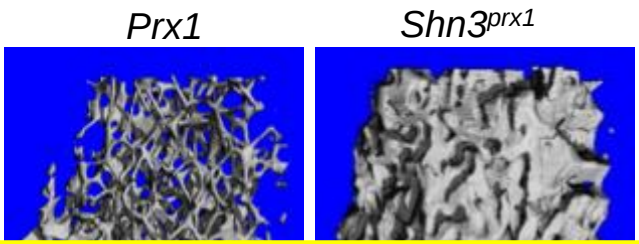
Normal bone mineral density distribution (increased with age)

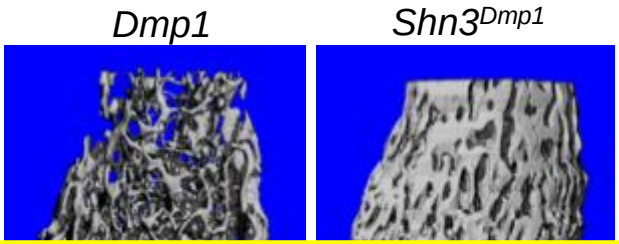
Cre-transgenic mice targeting different stages of osteoblast differentiation



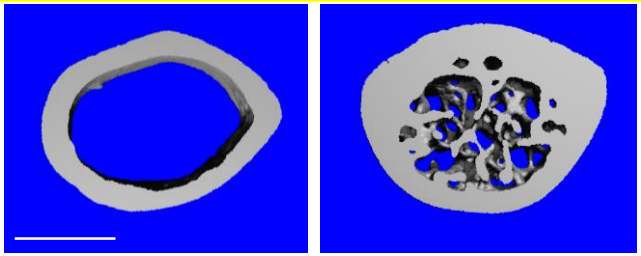
MSC: mesenchymal stem cell
OCP: osteochondral progenitor
OP: osteoprogenitor
OB: osteoblast
OC: osteocyte

Deletion of SHN3 in MSCs or in mature OB/osteocytes increases bone mass in mice

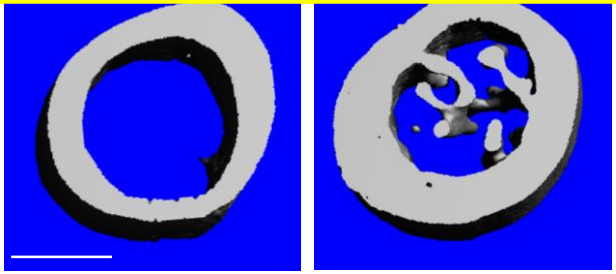




SHN3 function is intrinsic to osteoblasts

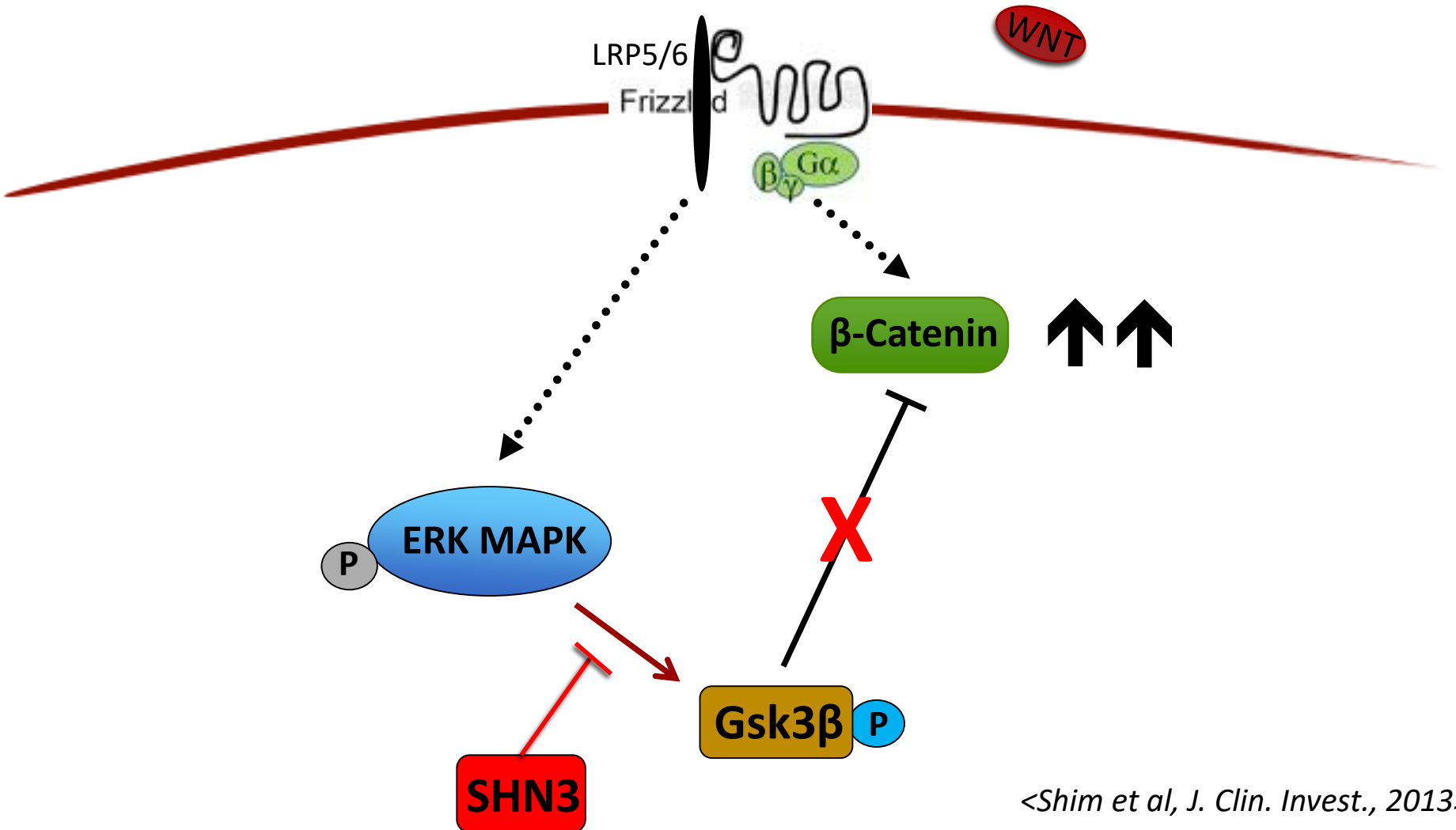


<Wein et al., PNAS, 2012>



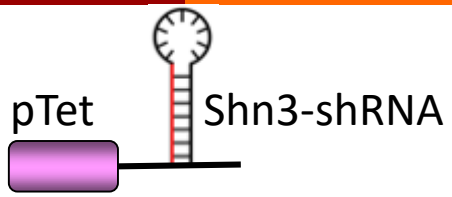
<Xu et al., Nat. Med, 2018>

SHN3 controls WNT signaling in osteoblasts

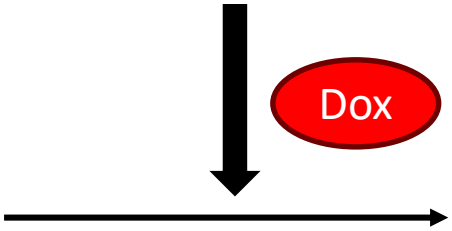


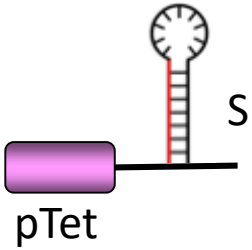
<Shim et al, J. Clin. Invest., 2013>

Transgenic mice with inducible knockdown of SHN3



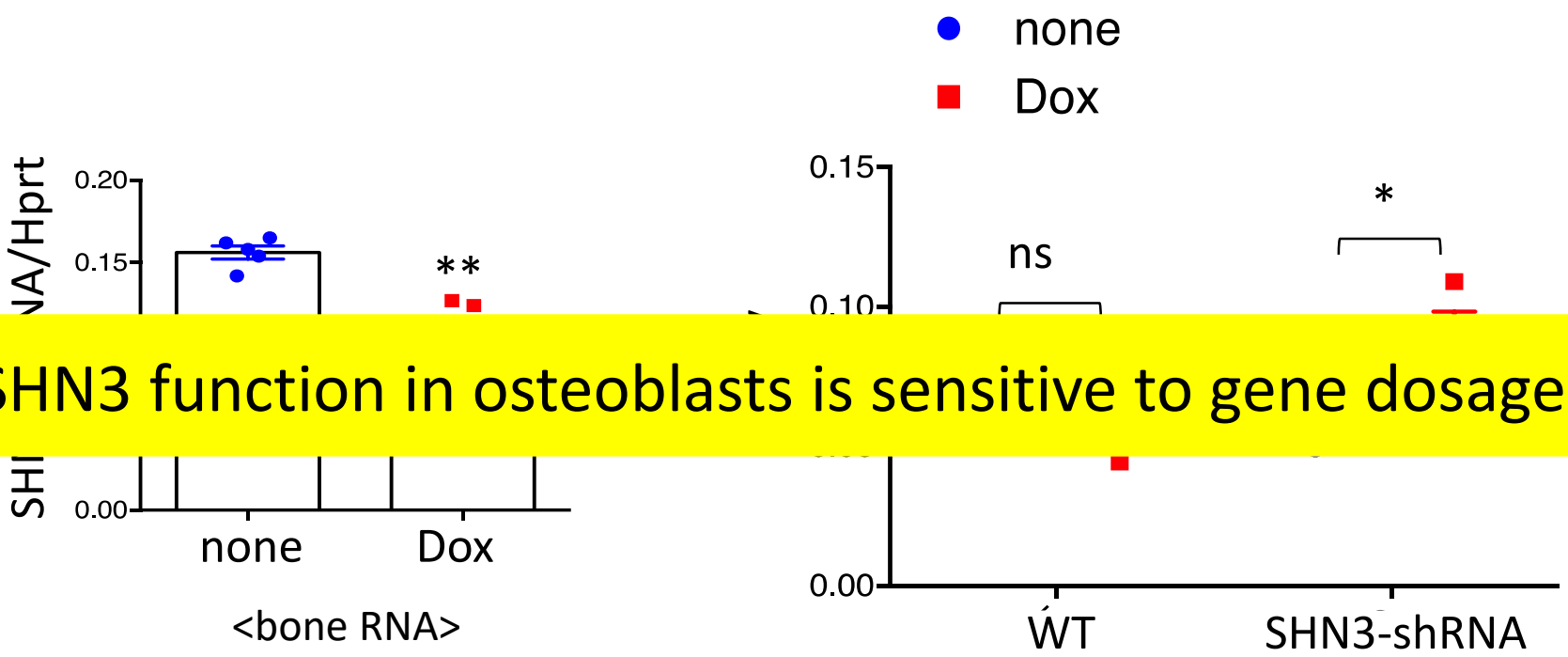
Doxycycline-containing food (6 week treatment)





SHN3-KD mice
(8 week old)

Short-term silencing of SHN3 in adult mice increases bone mass

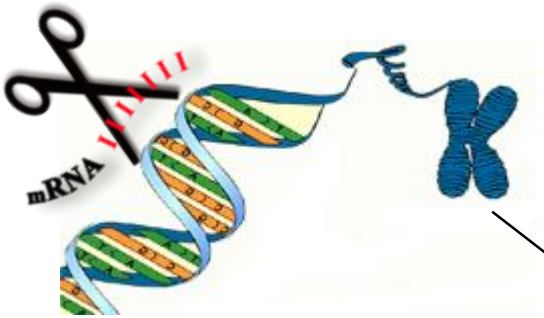


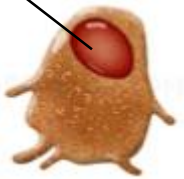
SHN3 function in osteoblasts is sensitive to gene dosage

<Shim et al, J. Clin. Invest., 2013>

Strategy to develop gene therapy for skeletal diseases

Healthy bone





osteoblast

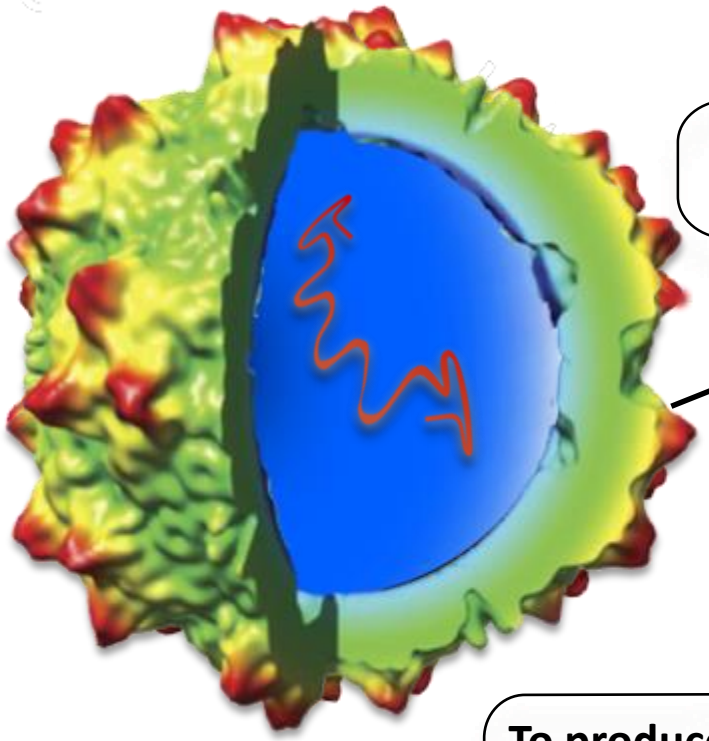
Bone-targeting probe

SHN3-gene silencer

Carrier

Osteoporotic bone

AAV-mediated delivery of gene silencer to bone

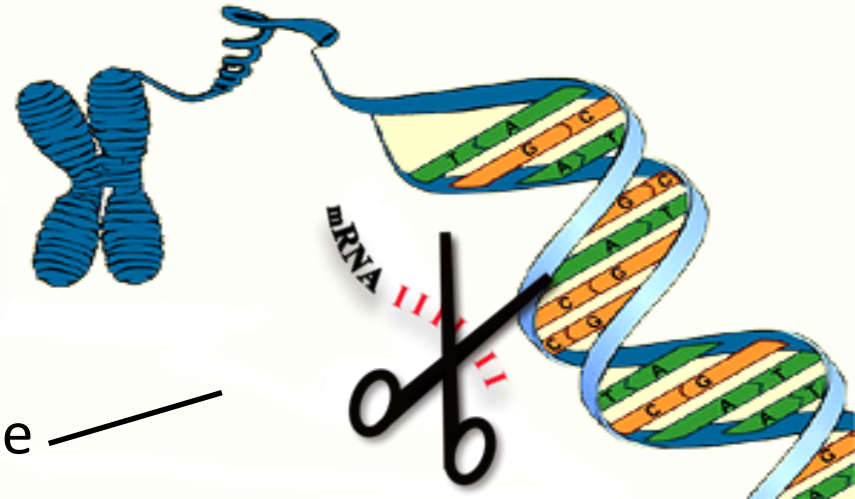


To guide where to go
- osteoblast in the skeleton

Capsid

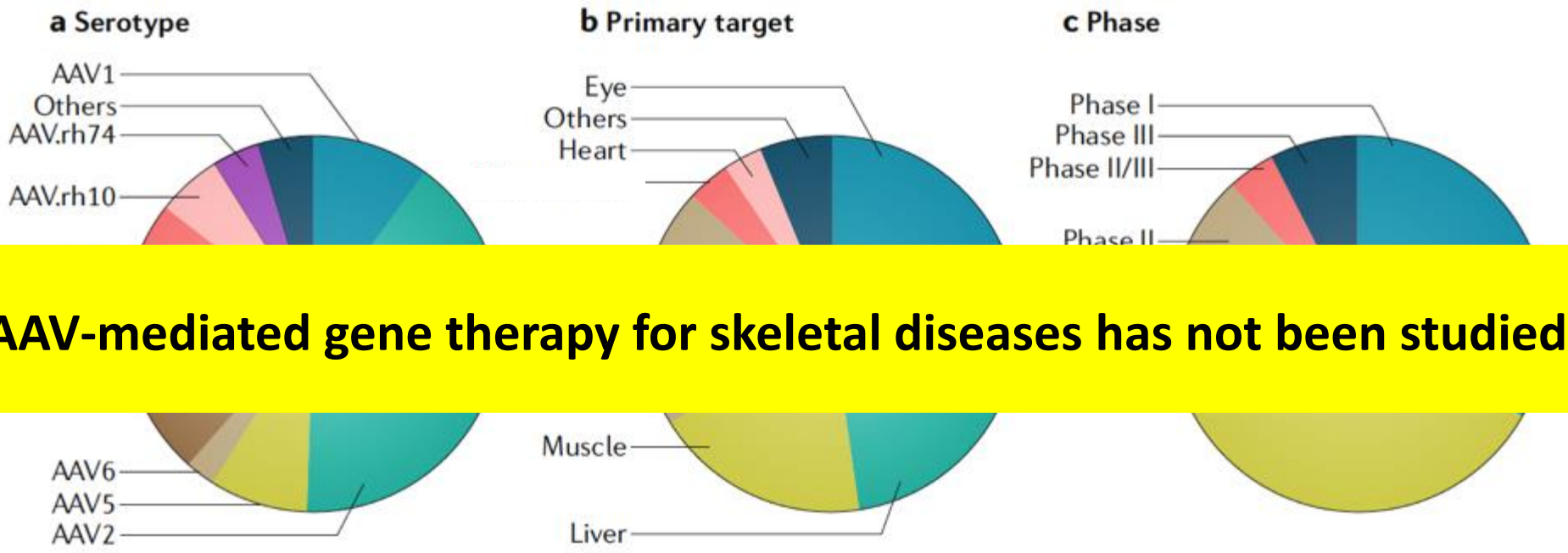
To produce active drug
- Gene silencing

Vector genome



AAV: adeno-associated virus

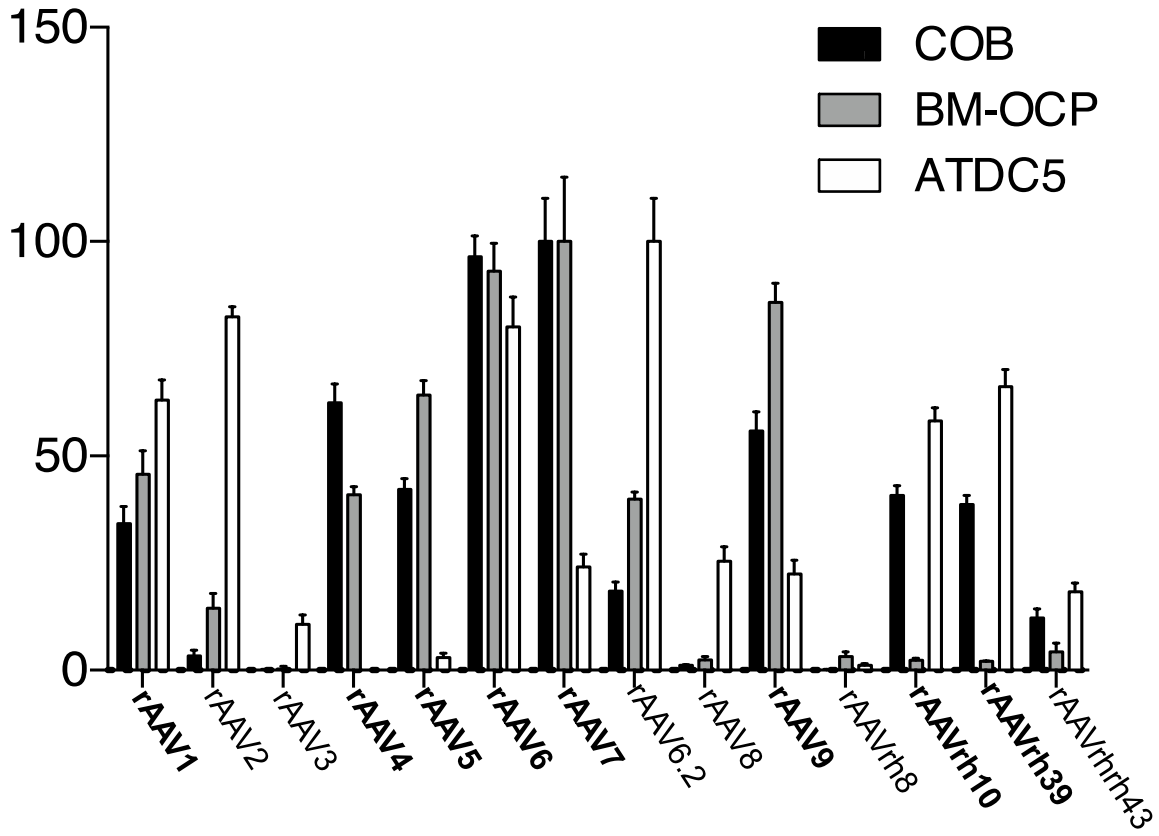
Current Gene Therapy Clinical Trials



AAV-mediated gene therapy for skeletal diseases has not been studied

Currently, 145 clinical trials have been registered at ClinicalTrials.gov

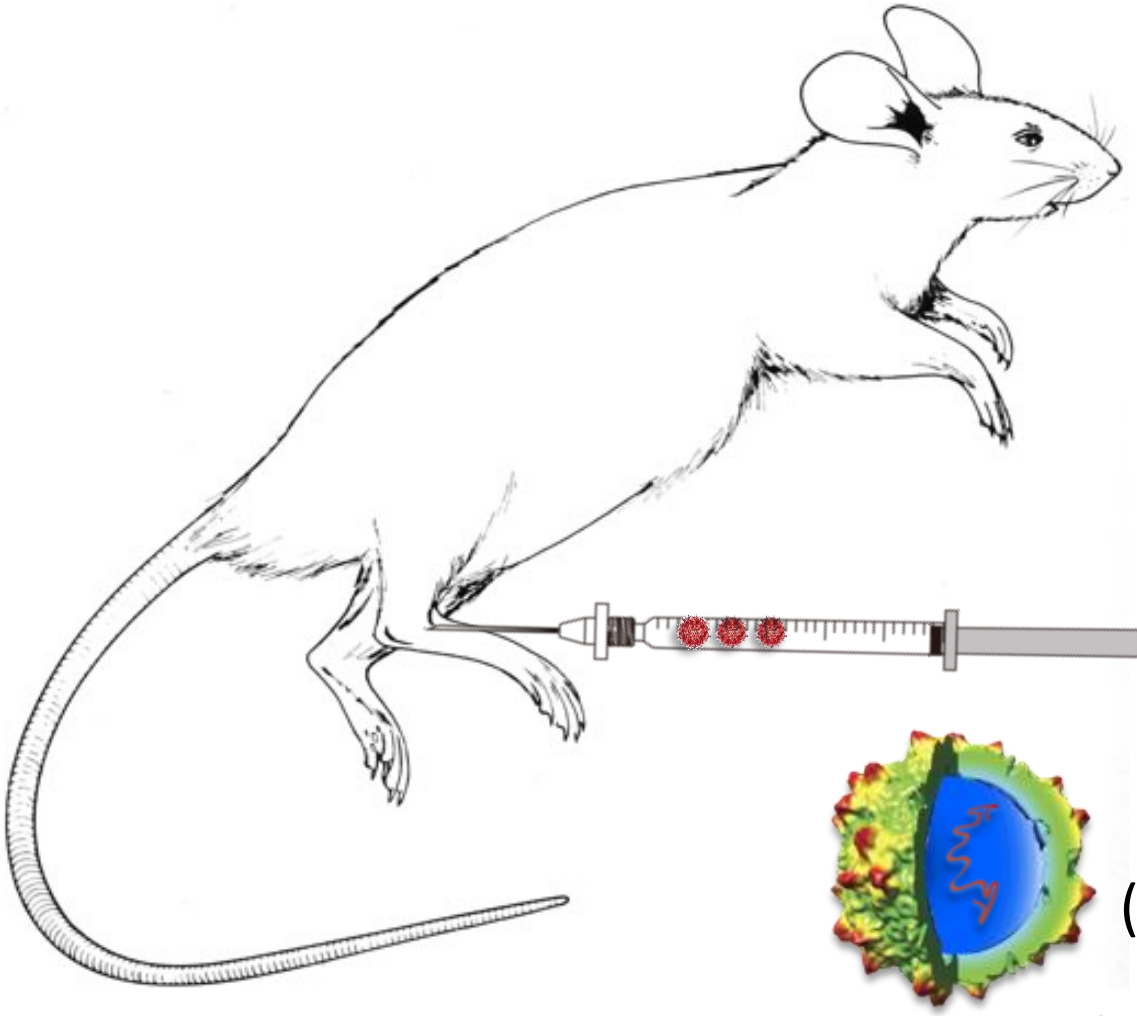
Identification of AAV serotypes that transduce bone cells (*in vitro*)



COB: calvarial osteoblast
BM-OCP: bone marrow-derived osteoclast precursor
ATDC5: chondrogenic cell line

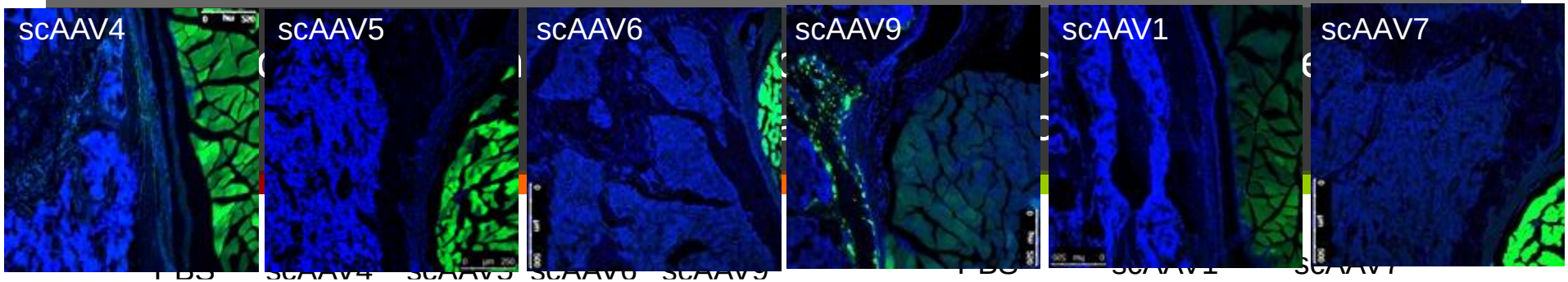
6 rAAV serotypes (AAV1, 4, 5, 6, 7, and 9) can transduce osteoblasts and osteoclasts

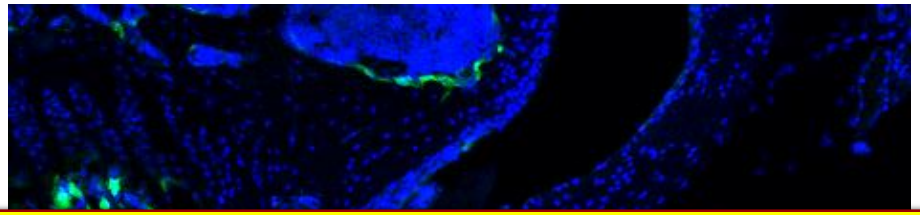
Identification of rAAV serotypes that can transduce osteoblasts and osteoclasts in the bone (*in vivo*)



Intraarticular injection

6 rAAV.*Egfp*
(AAV1, 4, 5, 6, 7, 9)

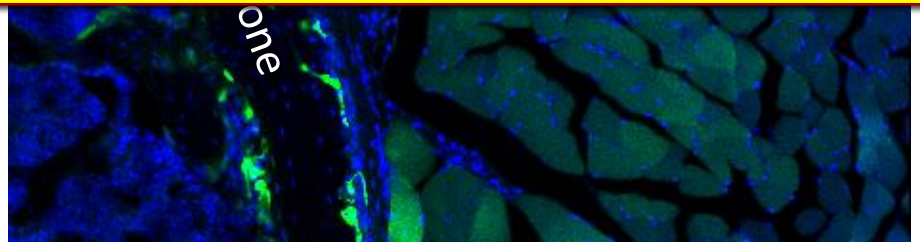




AAV9 is currently used in multiple human clinical trials

rAAV9-mediated gene transfer is safe in human (clinical phase 1)

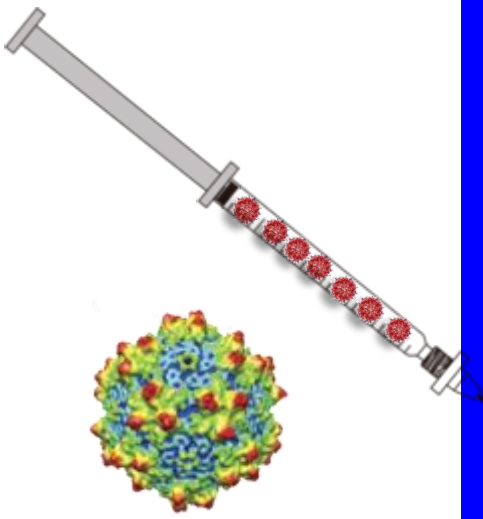
4. Sanfilippo syndrome type A (MPS IIIA) : Clinical phase 2/3, Sarepta Therapeutics
5. Mucopolysaccharidosis IIIB: Clinical phase 1/2, Abeona Therapeutics



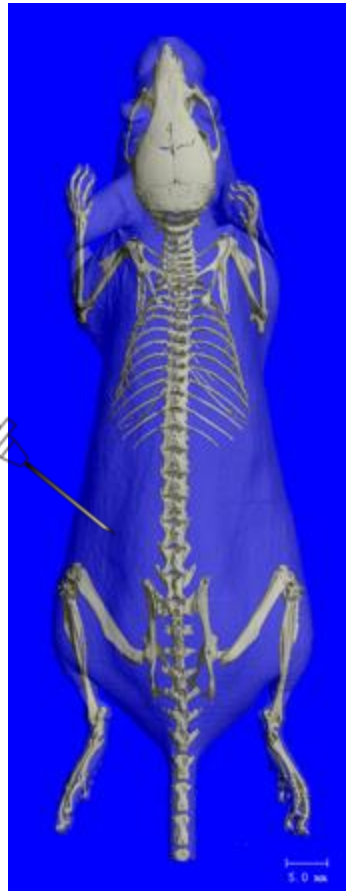
AC: articular cartilage

Systemically delivered rAAV9 transduces liver, heart, and skeletal muscle

Intravenous injection

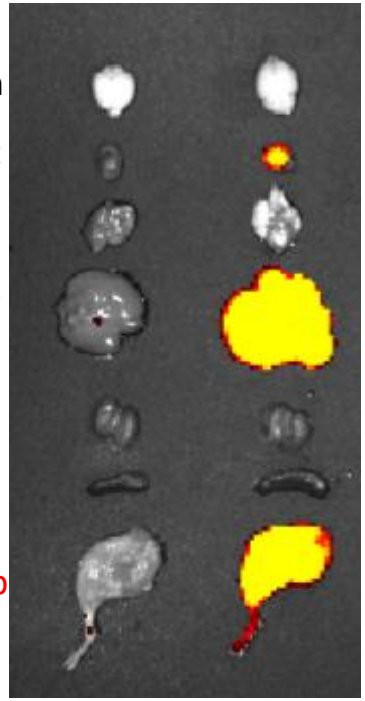


AAV9 (EGFP)

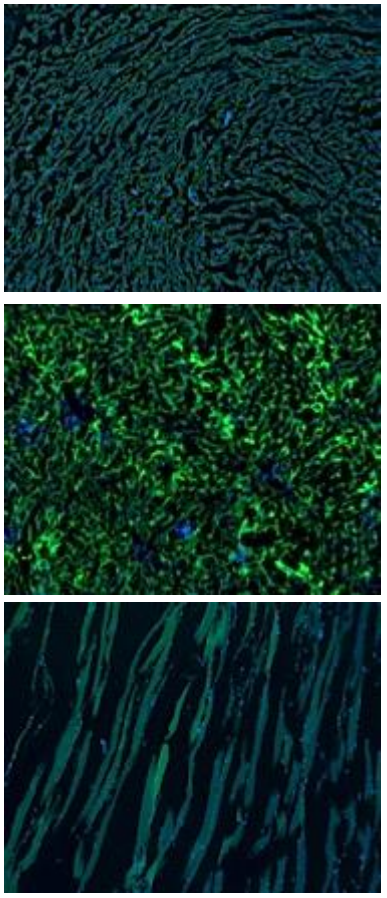


PBS AAV9-gfp

Brain
Heart
Lung
Liver
Kidney
Spleen
Hindlimb



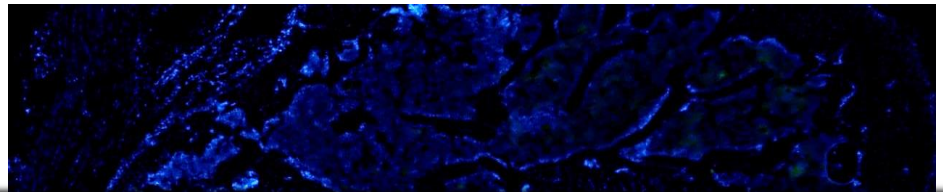
<IVIS-100 optical imaging>

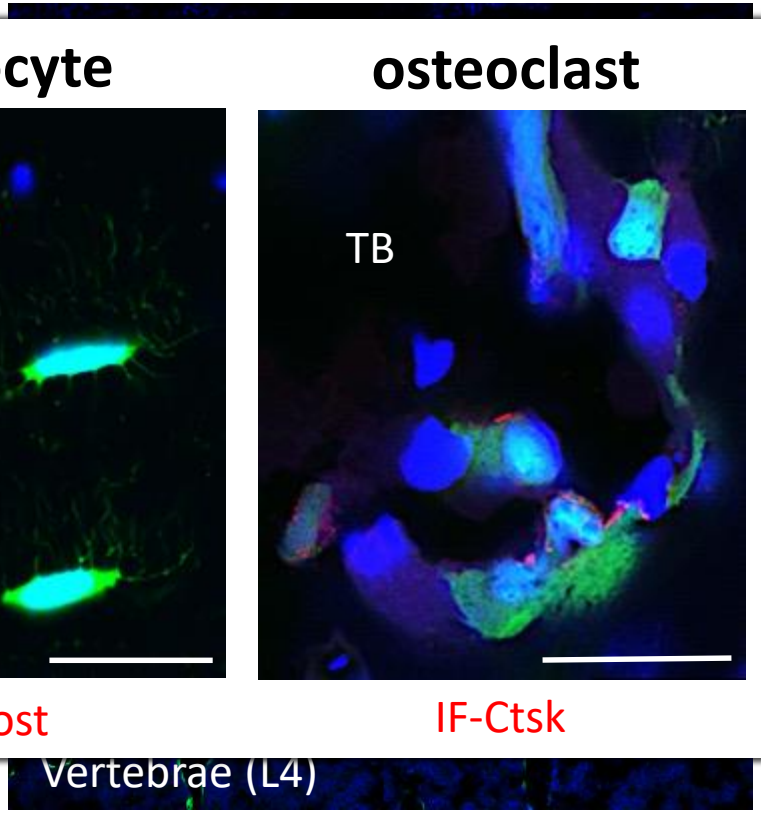


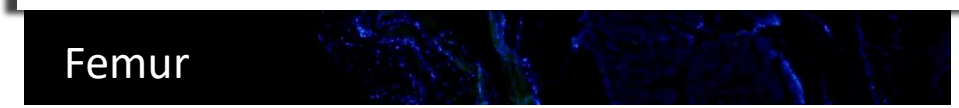
Heart
Liver
Skeletal muscle

<3 month old female mice 2 week post-injection>

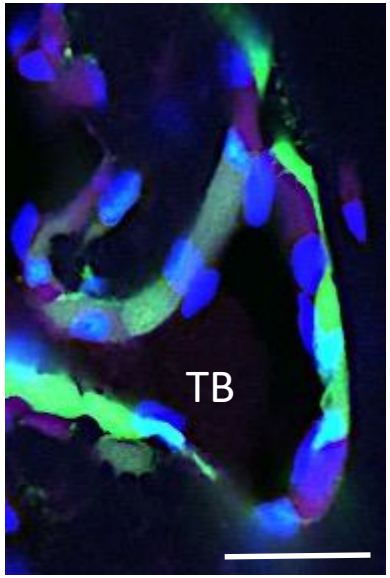
Systemic delivery of rAAV9 to skeleton in mice



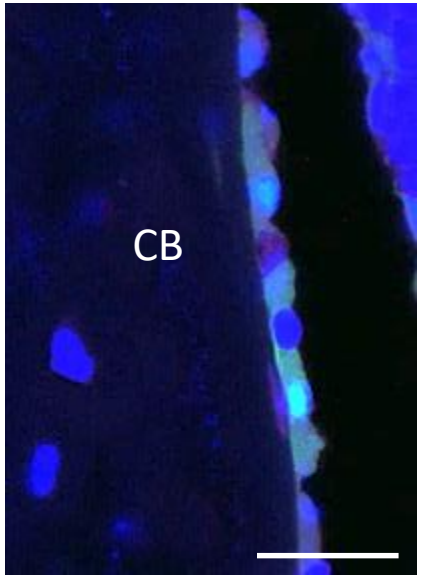




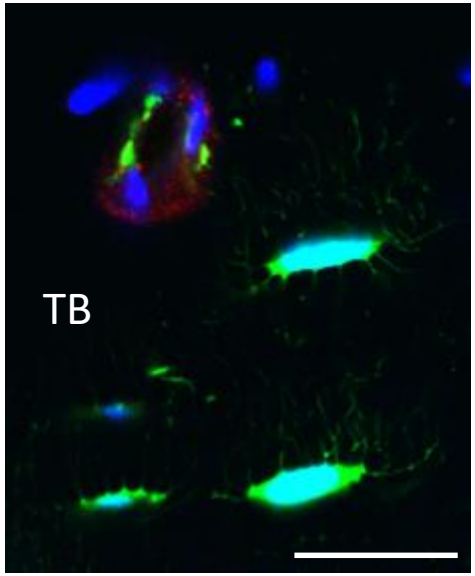
osteoblast **osteocyte** **osteoclast**



IF-Runx2



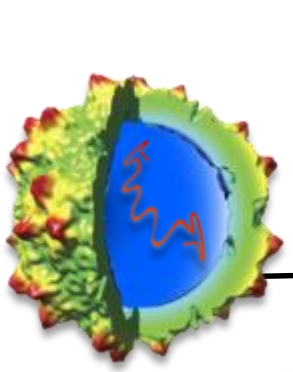
IF-Bglap



IF-Sost

IF-Ctsk

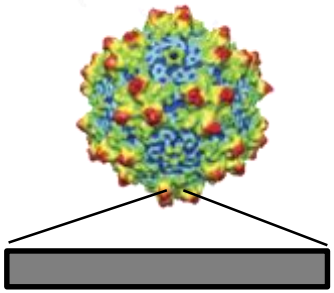
Development of bone-targeting AAV9



To guide where to go
- Osteoclast/osteoblast in the skeleton

Capsid

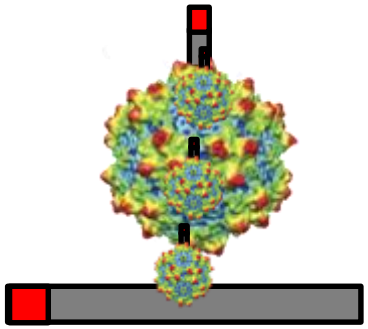
WT-AAV9



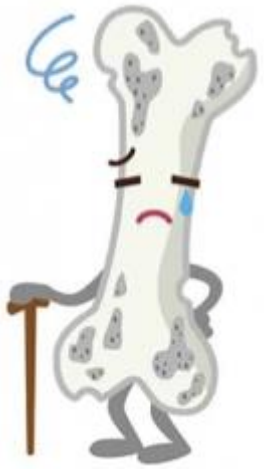
Capsid proteins

Genetic manipulation

BT-AAV9

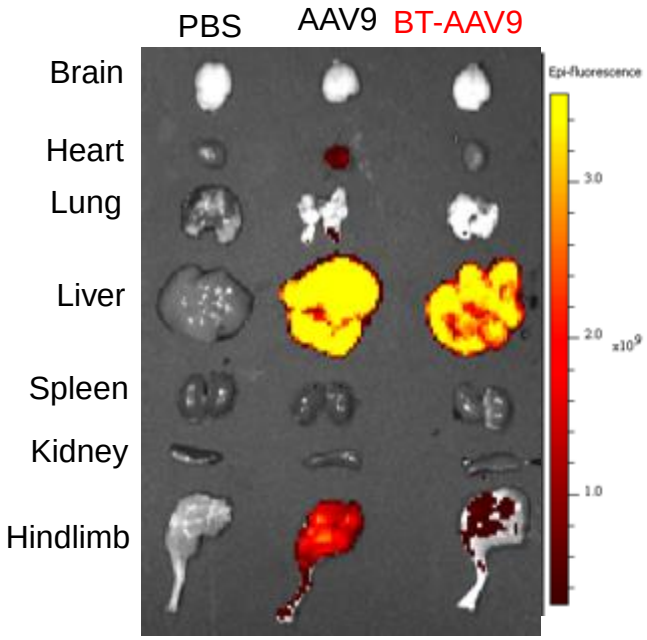


Bone-homing capsid (Grafting of DSS_6 peptides onto VP2)

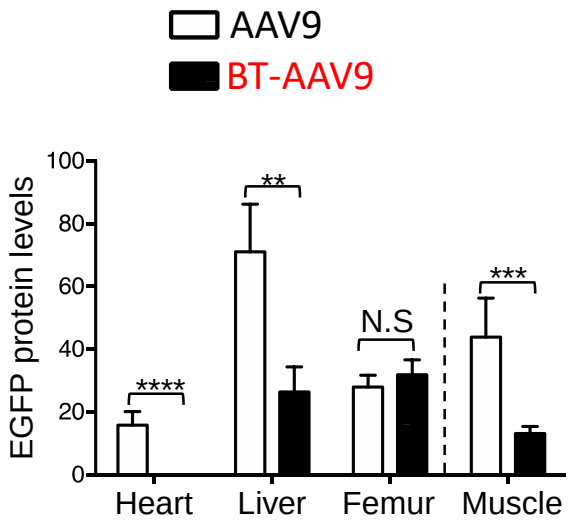


Osteoporotic bone

Bone-homing specificity is enhanced in BT-AAV9

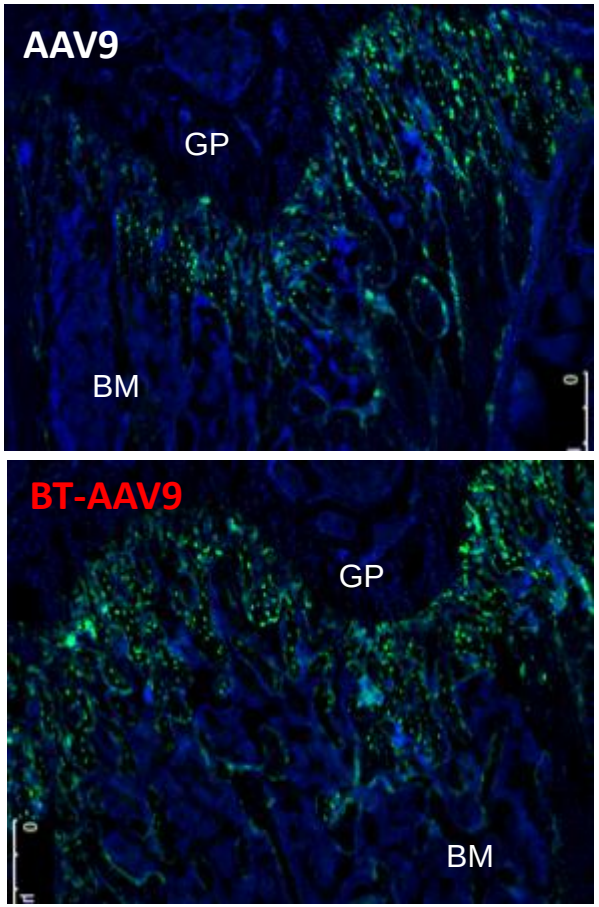


Optical imaging



WB: GFP

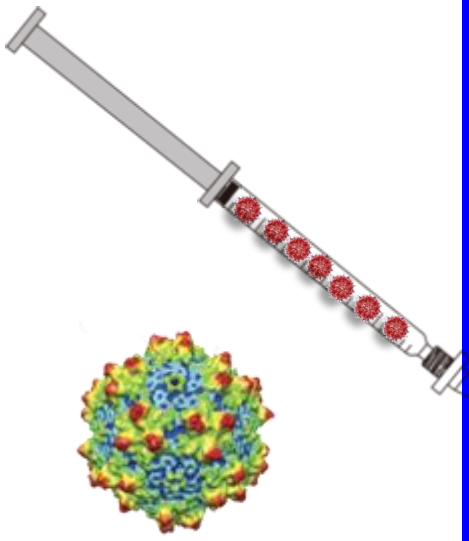
Femur



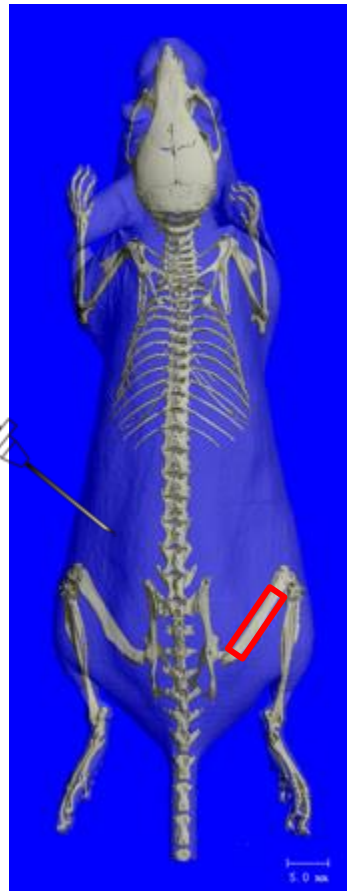
GP: growth plate
BM: bone marrow

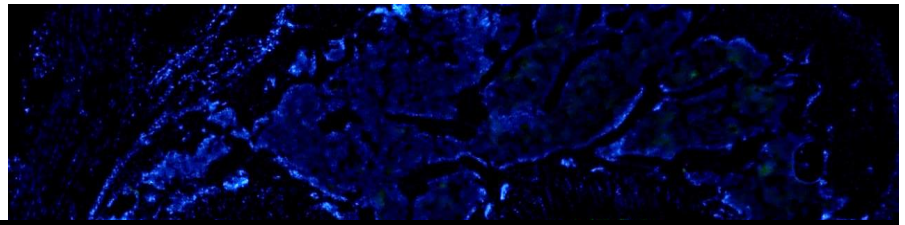
BT-AAV9 carrying SHN3 silencer targets osteoblast lineage cells following systemic delivery

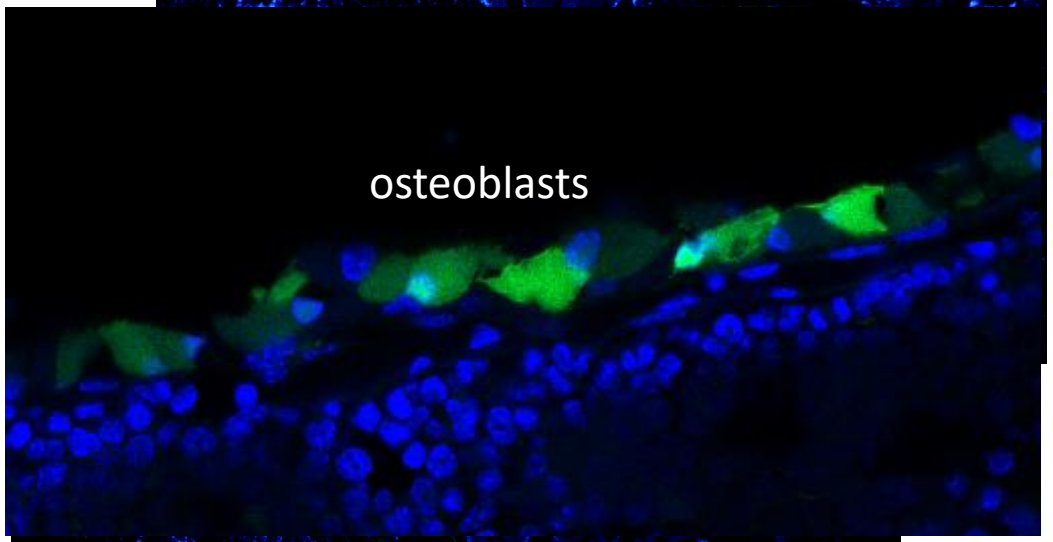
Intravenous injection

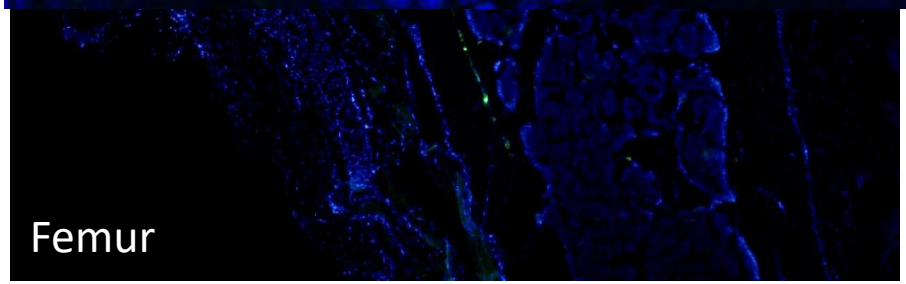


BT-AAV9
(amiR-SHN3)



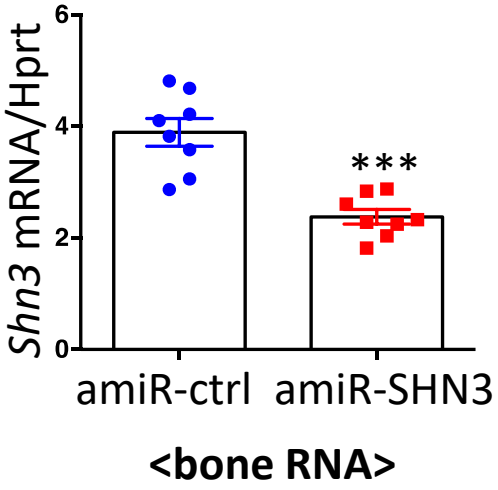


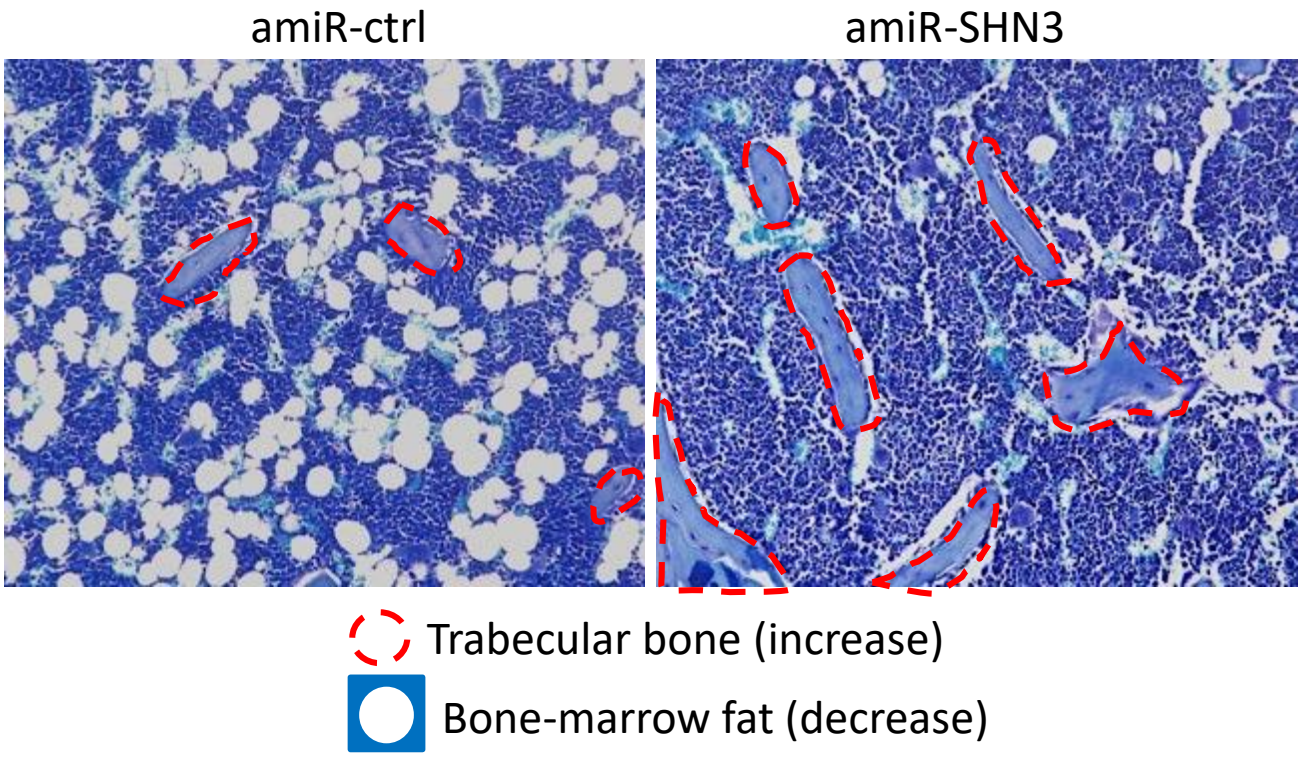




amiR: artificial miRNA

AAV-mediated silencing of SHN3 increases bone mass in mice

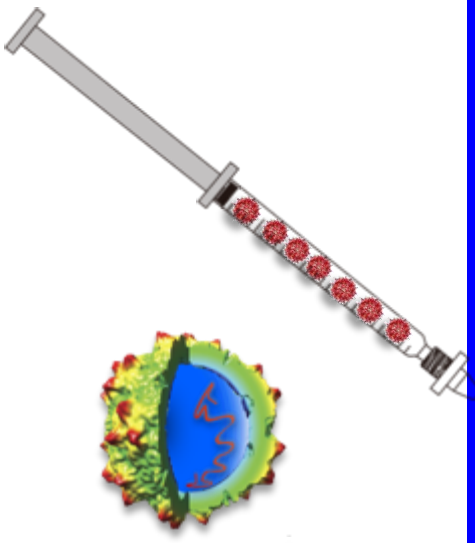




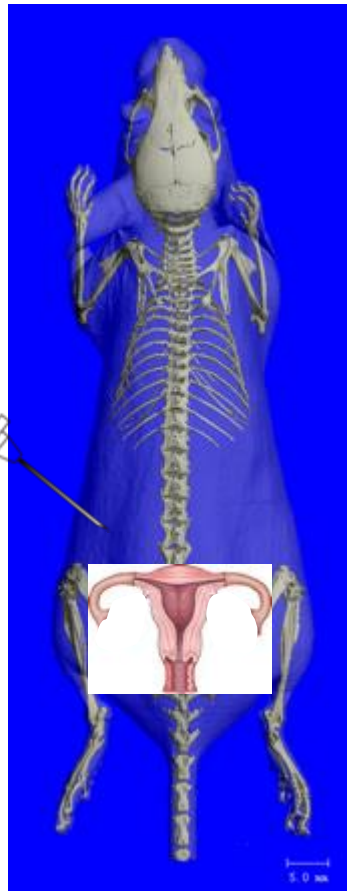
Mouse model of postmenopausal osteoporosis

(estrogen deficiency-induced osteoporosis in postmenopausal women)

intravenous injection



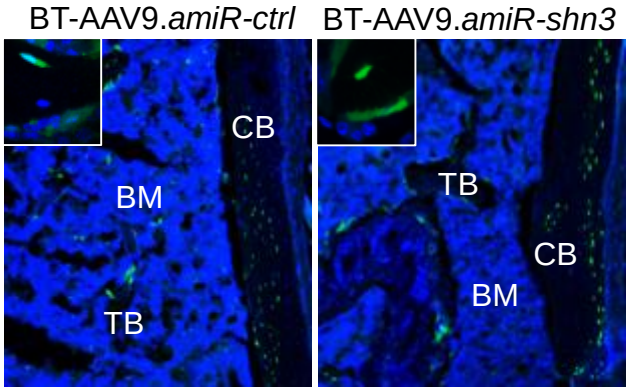
BT-AAV9
(amiR-SHN3)

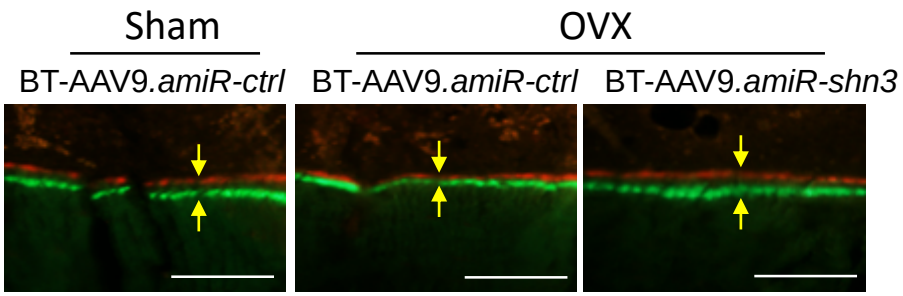


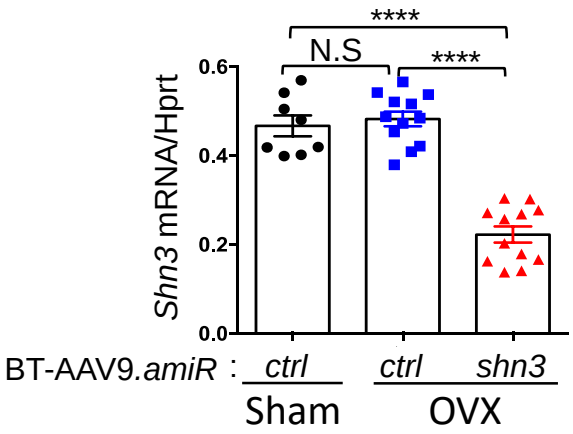
Ovariectomy

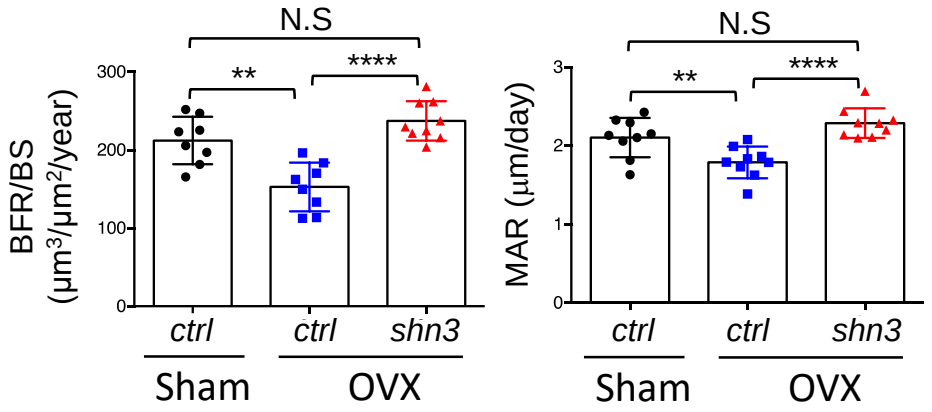
Estrogen-deficiency

BT-AAV9-mediated silencing of SHN3 promotes bone formation in postmenopausal osteoporosis



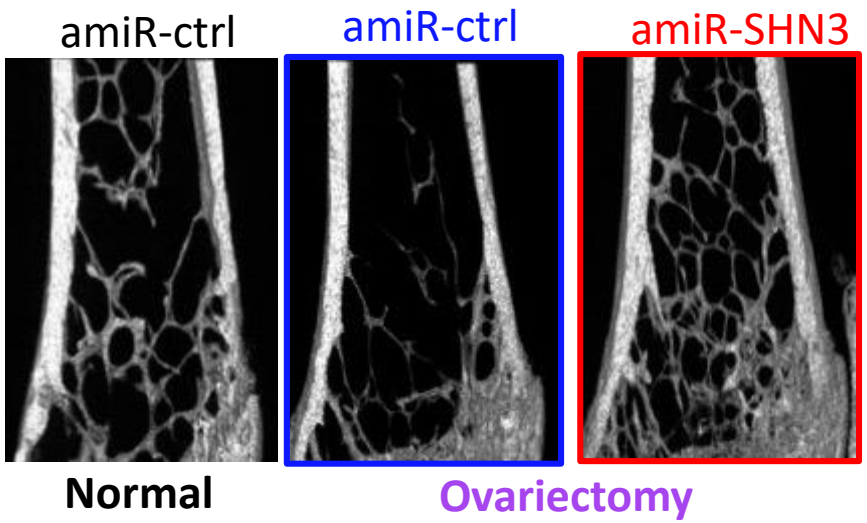


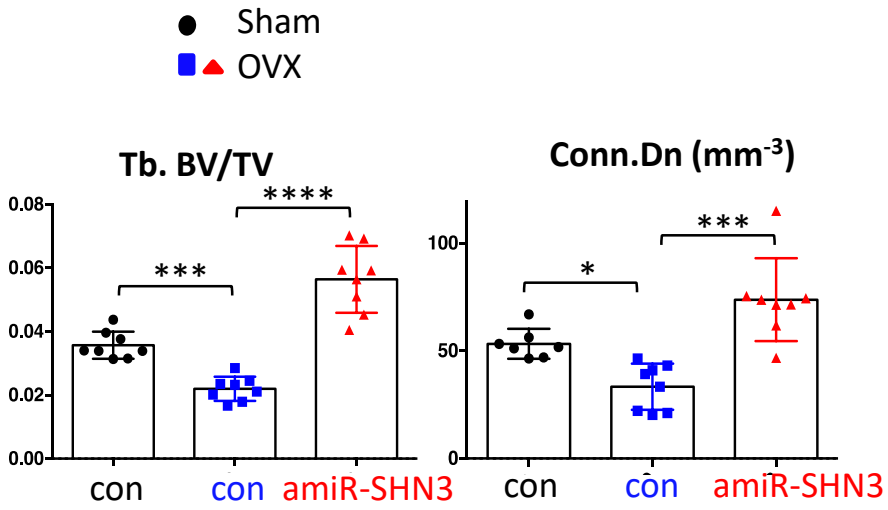




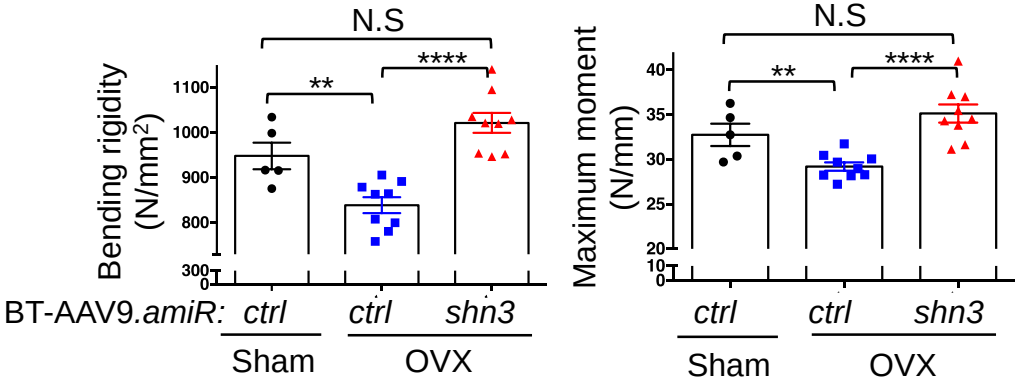
<Dynamic histomorphometric analysis on the femoral metaphysis>

BT-AAV9-mediated silencing of SHN3 reverses bone loss in postmenopausal osteoporosis

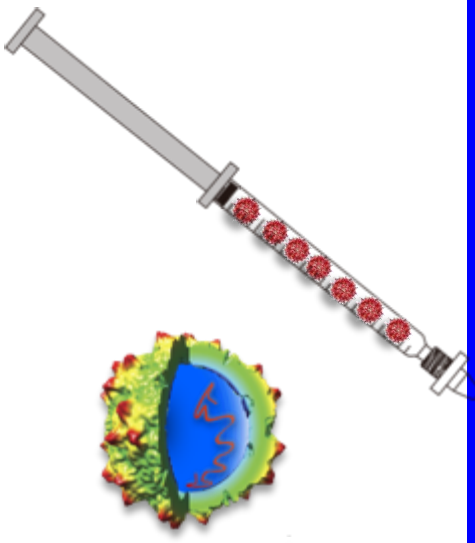




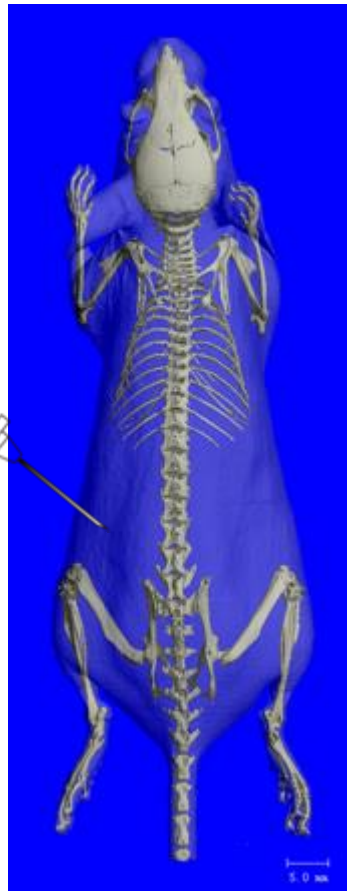
<Three-point bending test>



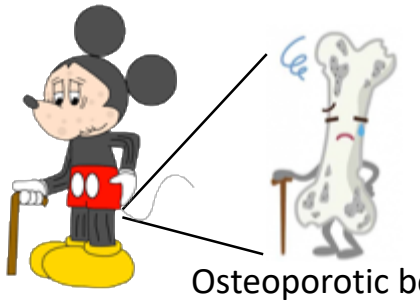
Mouse model of aging-associated osteoporosis



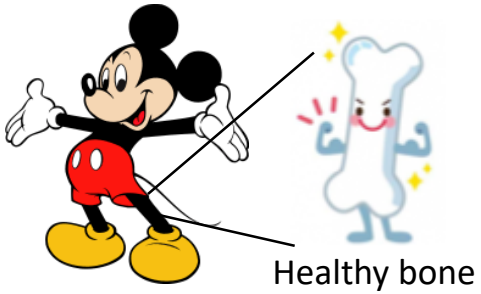
BT-AAV9
(amiR-SHN3)



<18 month old male>

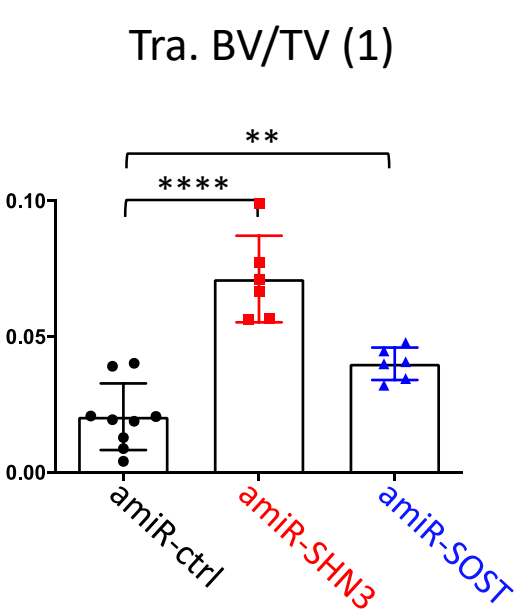


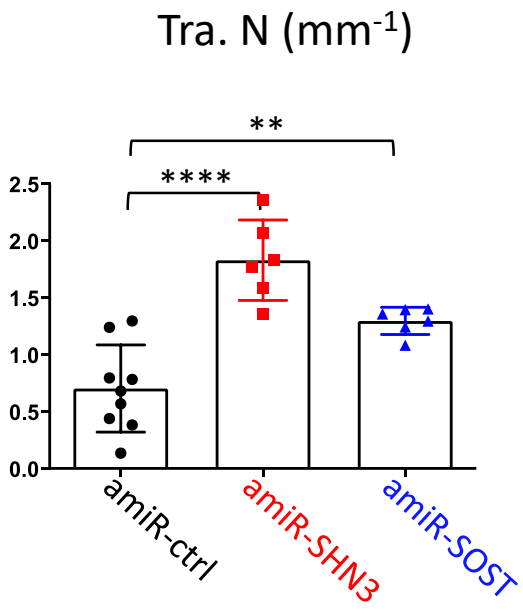
Osteoporotic bone

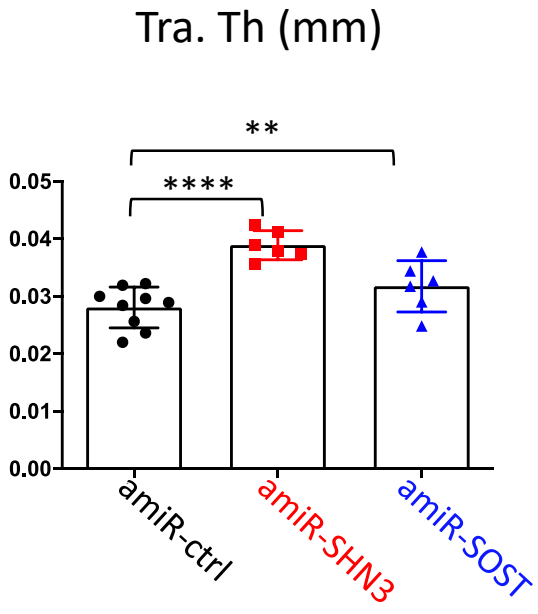


Healthy bone

AAV-mediated gene therapy prevents aging-associated osteoporosis

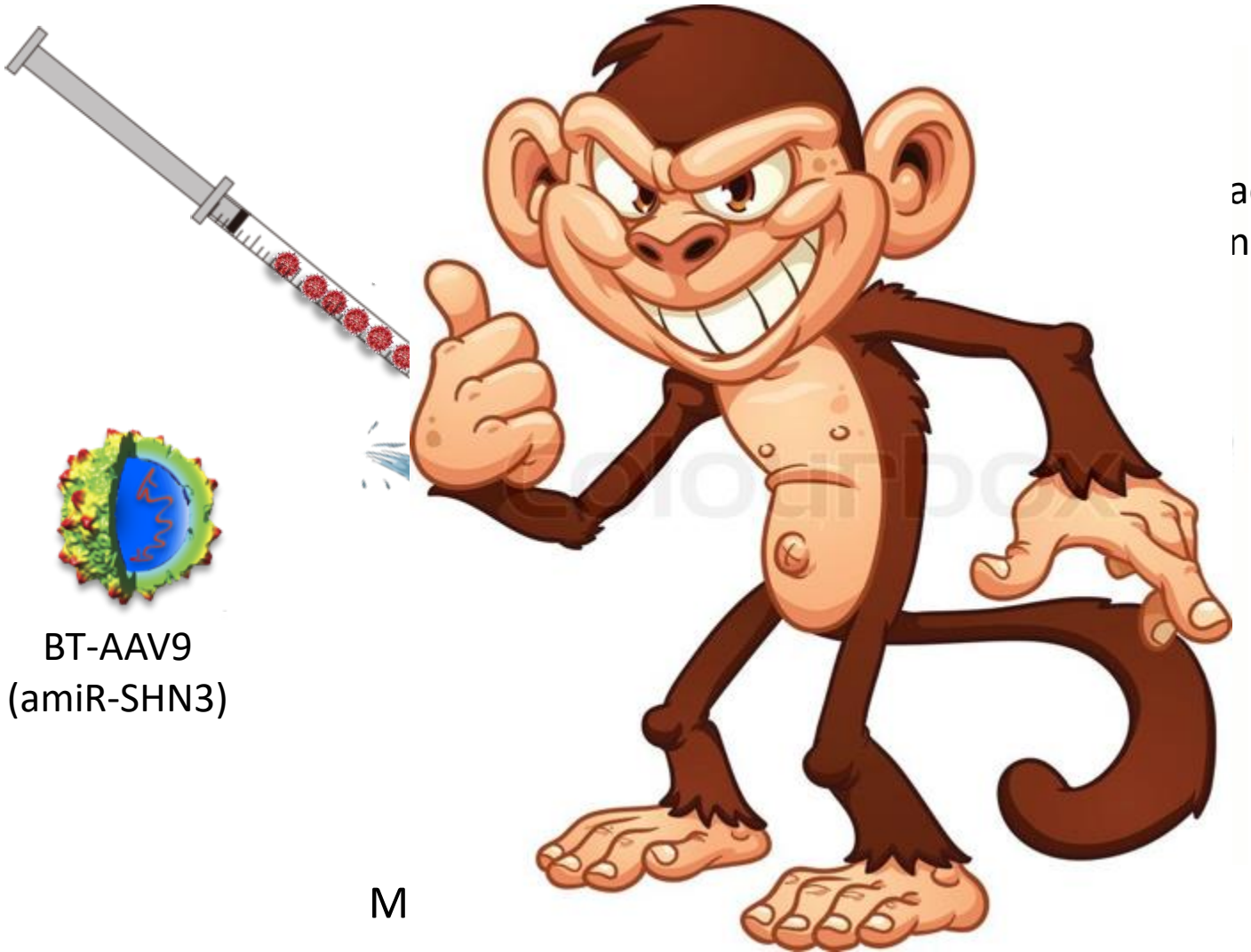






Romosozumab (Evenity): humanized anti-sclerostin antibody (FDA-approval, 2018)

Therapeutic effects of BT-rAAV9.amiR-SHN3 on large animals with osteoporosis



BT-AAV9
(amiR-SHN3)

acture
ne mass

M

Acknowledgement

Shim lab

Yeon-Suk Yang
Jungmin Kim
Zheni Stavre
Chujiao Lin
Sachin Ramdas
Aijaz Bhat
Wontaek Oh
Oksun Lee
William Song
Moulika Bandaru

Gao lab

Jun Xie
Phillip Tai
Dan Wang

Bone Analysis Core

Viral Vector Core

AAVAA Therapeutics

JiHea Kim
Yeon-Suk Yang
Wontaek Oh

Brigham Women's Hospital

Dr. Ellen Gravallese

Weill Cornell Medicine

Dr. Ching Tung
Dr. Seung-Koo Lee

Yonsei Severance Hospital

Dr. Kwanhwan Park
Dr. Suk-Kyo Seo

KAIST

Dr. Hyonho Chun

NIH-R01 AR068983, R21 AR072836, R21 AR073331

SRAs: Glory Harvest Group and AAVAA Therapeutics