Bone Loss After SCI: What Do Animal Studies Tell Us About the Cause and Potential Treatments

Christopher P. Cardozo, MD
Director, Molecular Program, COE for the Medical Consequences of SCI
James J. Peters VAMC, Bronx, NY
Associate Professor, Mount Sinai School of Medicine, New York, NY

Disclosures

- Christopher Cardozo, MD
  Grants/research support:
  VA Research and Development Service Grants B4162C, B4616R, and F6997R and the DOD.

- CME Staff Disclosures
  Professional Education Services Group staff have no financial interest or relationships to disclose.

Learning Objectives

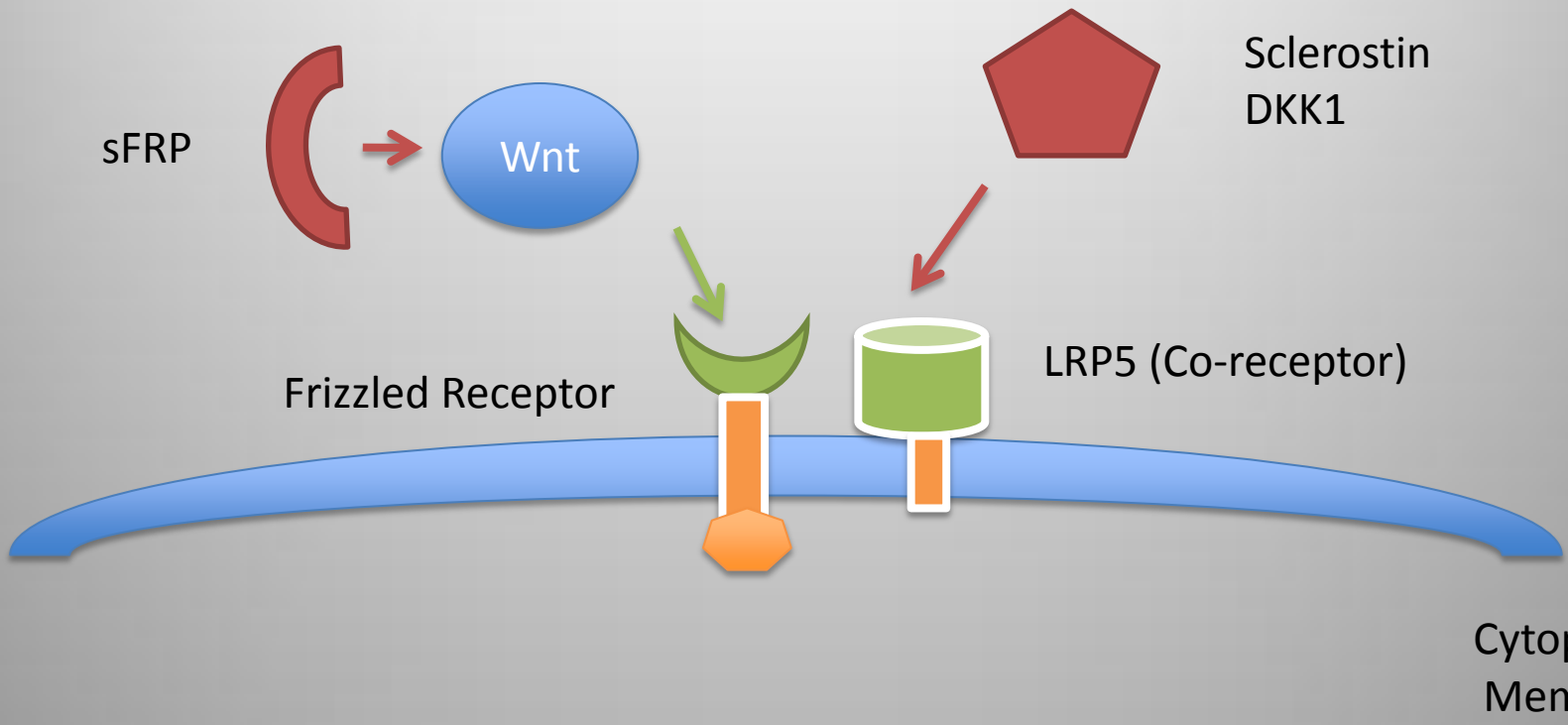
At the conclusion of this activity, the participant will be able to:

- A. Discuss the key changes in molecular signals pertinent to bone loss after SCI.
- B. Discuss the potential role of androgens in treatment of bone loss after SCI suggested by findings from animal studies.
- C. Discuss acute benefits to bone health of mechanical reloading of bone by FES that are suggested by animal studies.
- Identify two potential targets for drugs in bone loss after SCI.

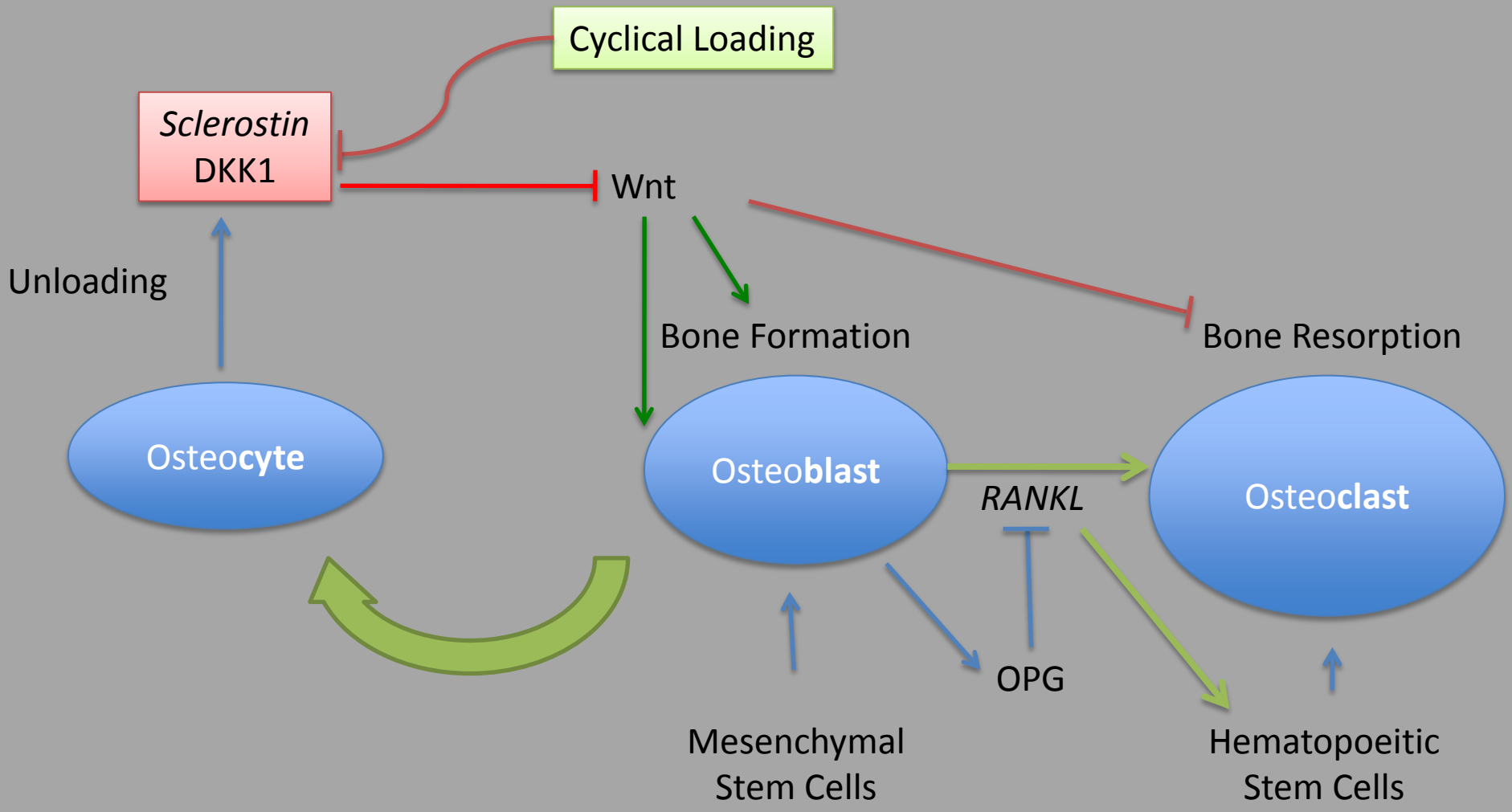
Utility of Animal Models

- Examine the temporal sequence of changes in bone mass and structure
- Through genetic manipulations, determine the underlying cellular and molecular events
- Evaluate candidate therapies

Wnt Signaling (Canonical)



Key Elements in Bone Biology

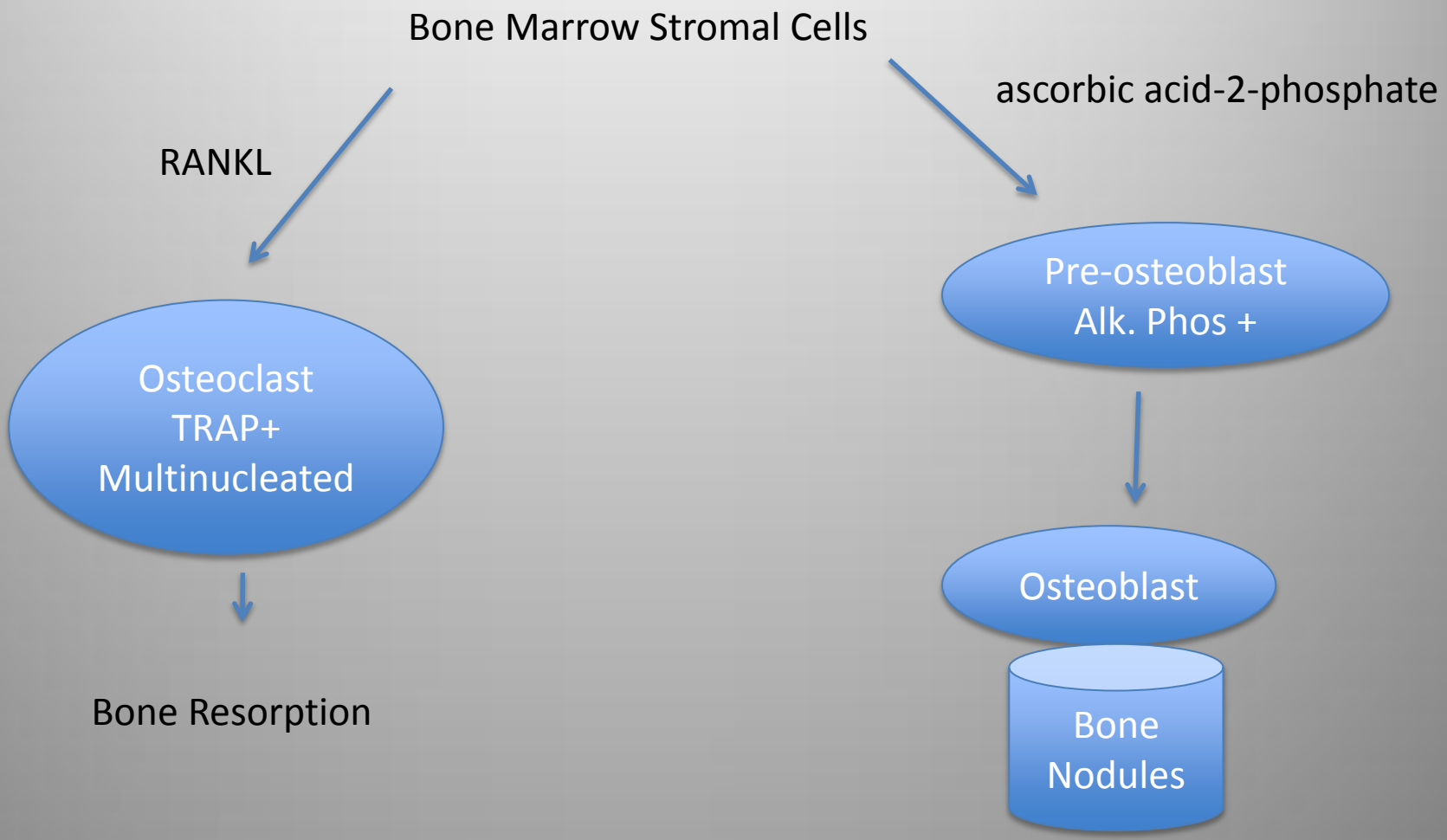


Effect of Unloading on SOST/Sclerostin

Robling et al. 2008 J Biol Chem 283:5866-75/

- In the rat ulna, mechanical loading reduced SOST and increased bone formation rates.
- These changes correlated with loading and strain gradients.
- Unloading increased SOST expression in-vivo at 3 and 7 days.

Ex-vivo Culture of Marrow Cells



Influence of Unloading on Bone

Grano et al. 2002 Calcif Tissue Int 70:176.

- Male rats had hindlimbs unloaded for 5 days by tail suspension.
- Osteoblast numbers in bone and bone formation rate were reduced by 34% and 39%.
- Numbers of osteoblasts present in ex-vivo cultures of bone marrow stromal cells was reduced and these OB exhibited reduced bone forming activity.
- Unloading stimulated increased IL-6 release from bone marrow stromal cells.

Features of Bone Loss in a Rat Model of Incomplete SCI

Morse et al. 2008 Osteoporosis Int 19:645-52.

- Male Rats (juvenile) with a severe (10gx50 mm) contusion injury studied at 10 days after SCI
- BMD at the proximal metaphysis reduced by 34%.
- 3-fold increase in osteoclast numbers at the growth plate.
- There appeared to be a reduction in bone formation rate at the distal metaphysis and a mineralization defect of newly formed bone.
- Thinning and disorganization of chondrocytes was noted at the growth plate.

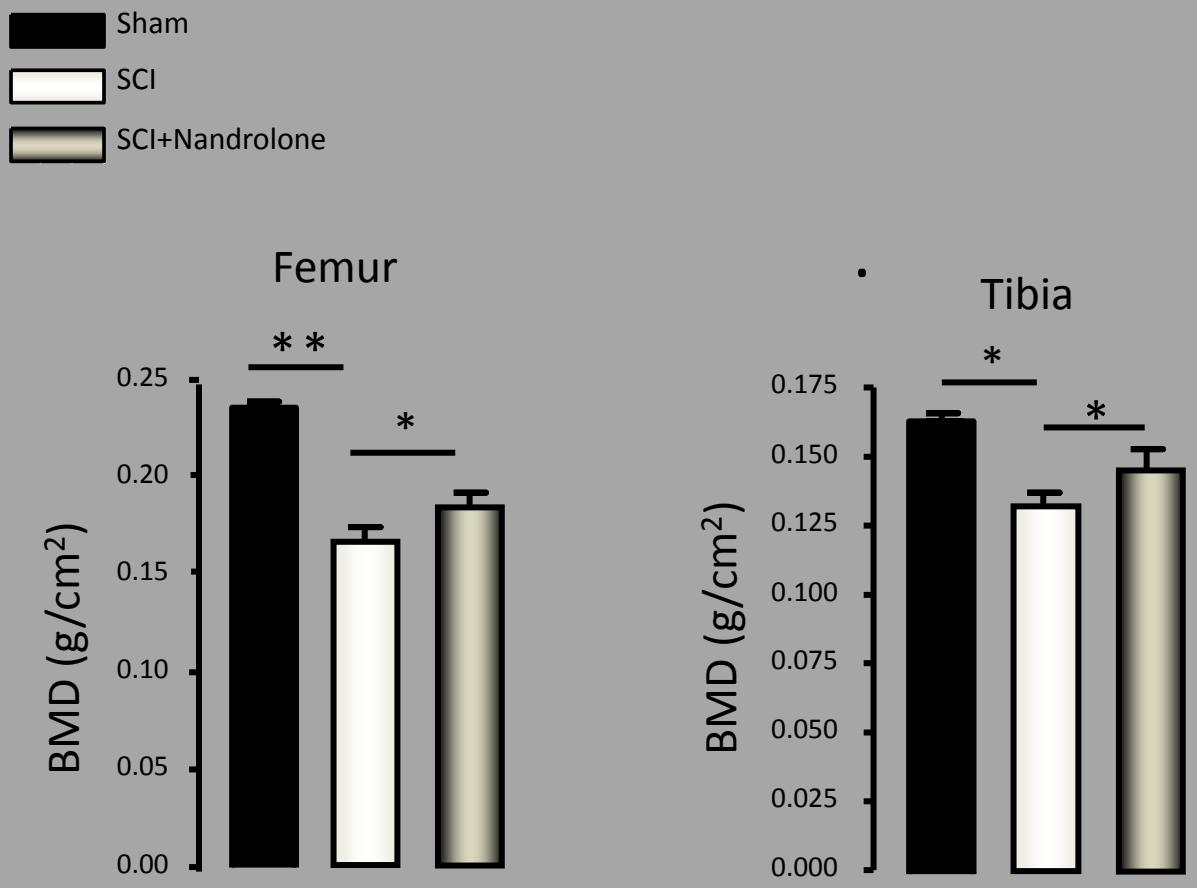
Evidence that Androgens Influence Bone

- Hypogonadism is common after SCI (Tsitouras, et al. 1995 *Horm Metab Res*, 27(6), 287-92).
- Studies of AR-knockout or gonadectomized mice showed impaired bone mineralization, accelerated bone resorption and worsened bone loss due to unloading (Chiang et al. J Bone Miner Res 2009; **24**(4)**:** 621-31; Li et al. Bone 2009; **45**(4)**:** 669-76; De La Piedra *et al. Aging Male* 2010).
- Nandrolone reversed bone loss due to microgravity (Wimalawansa et al. *J Appl Physiol* 1999; **86**(6)**:** 1841-6.)
- Nandrolone attenuated bone loss after nerve transection (Cardozo et al. Ann N Y Acad Sci 2010; **1192**(1)**:** 303-6)

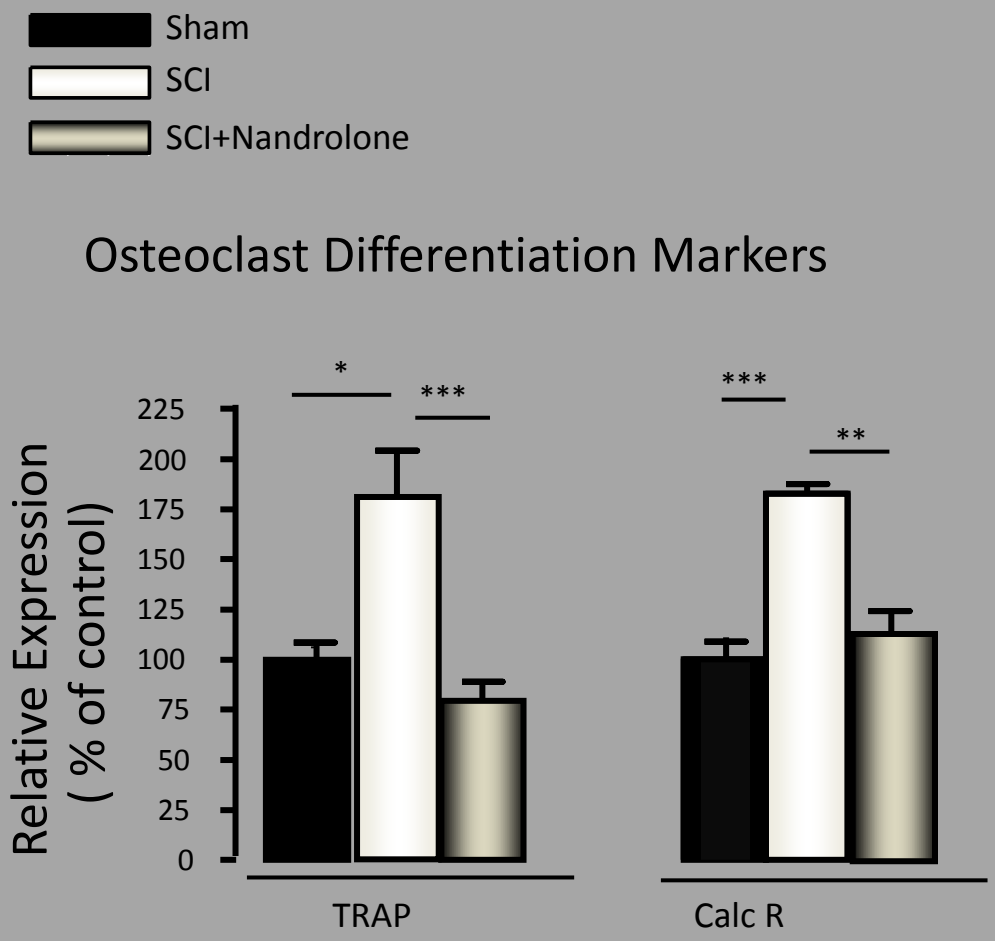
Animal Model

- Male Wistar rats with complete transection at T9-T10.
- Nandrolone plus testosterone (replacement dose) administered beginning day 29 after SCI and continued through day 56.
- Bones harvested for analysis at day 56.

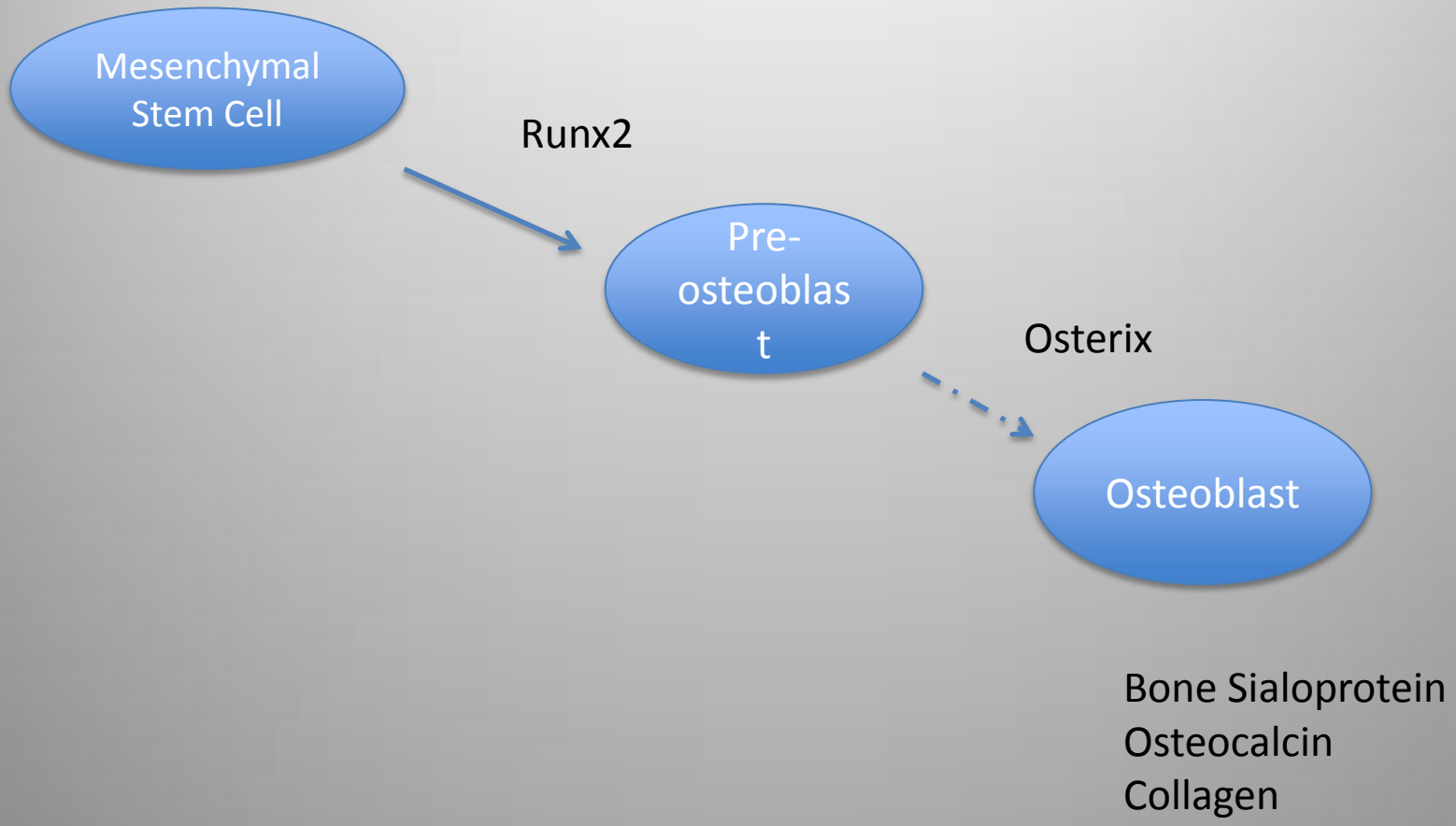
Nandrolone Reduces Bone Loss After SCI



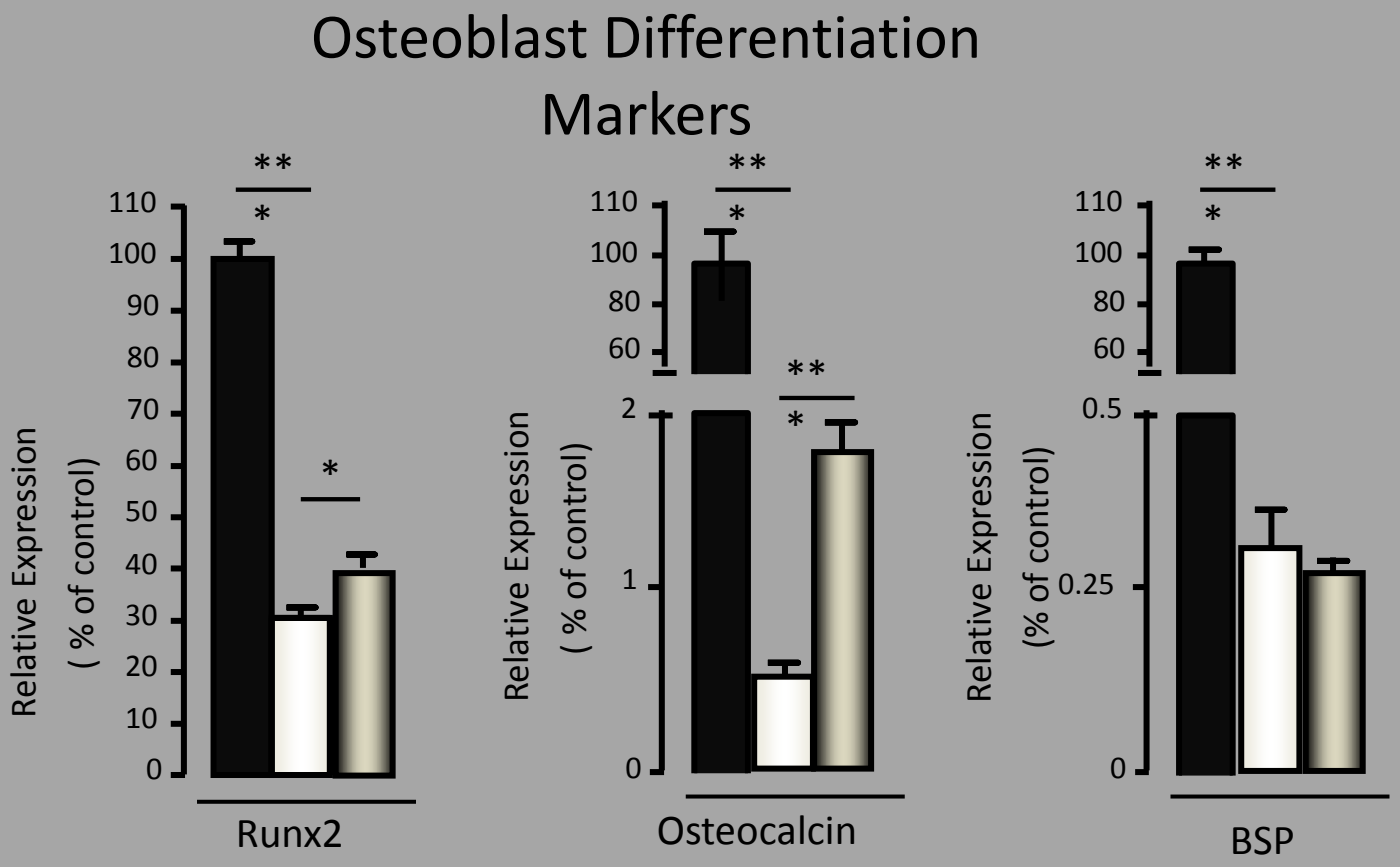
Nandrolone Reversed Upregulation of Osteoclast Markers after SCI



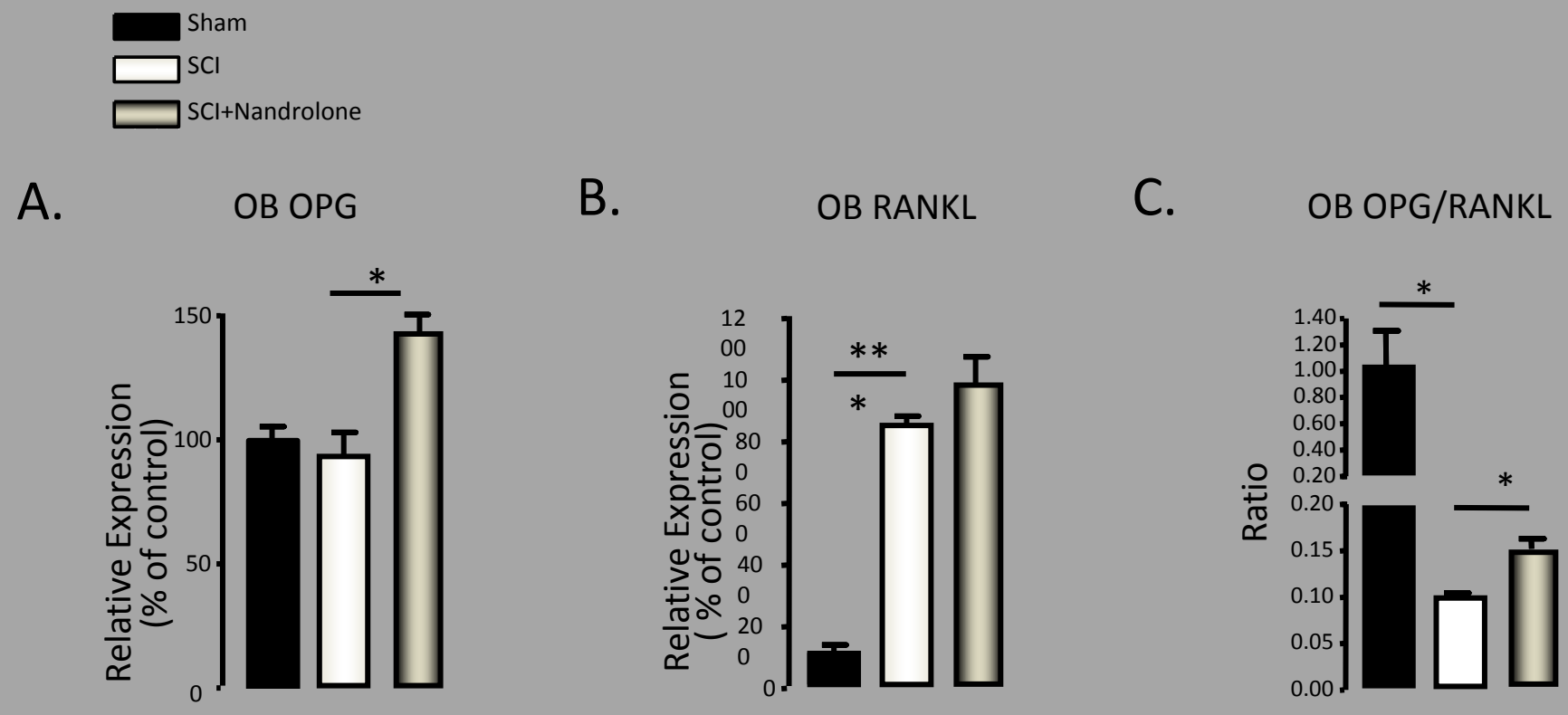
Differentiation Pathway of Osteoblasts



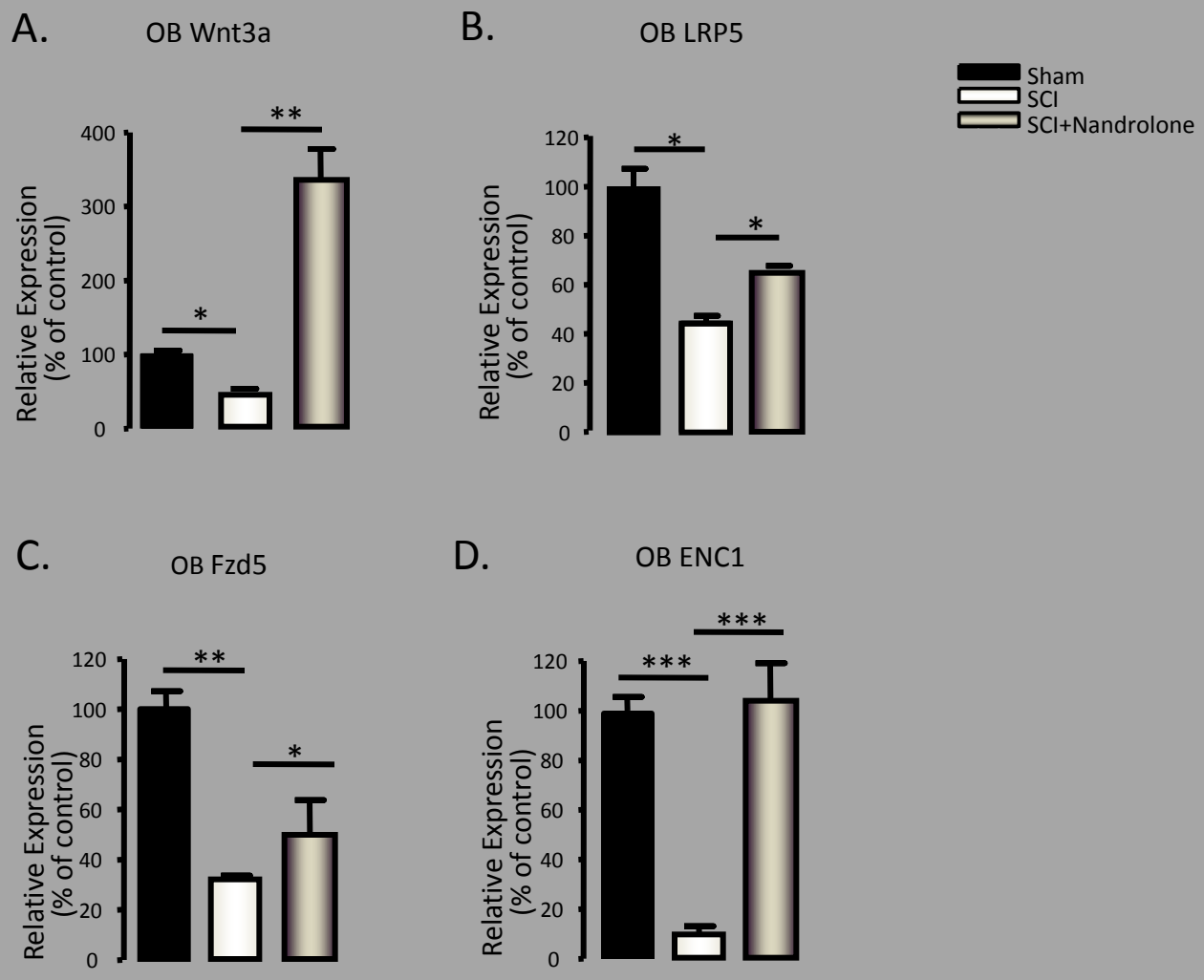
Nandrolone Partially Reversed Reductions in Osteoblast Differentiation Markers



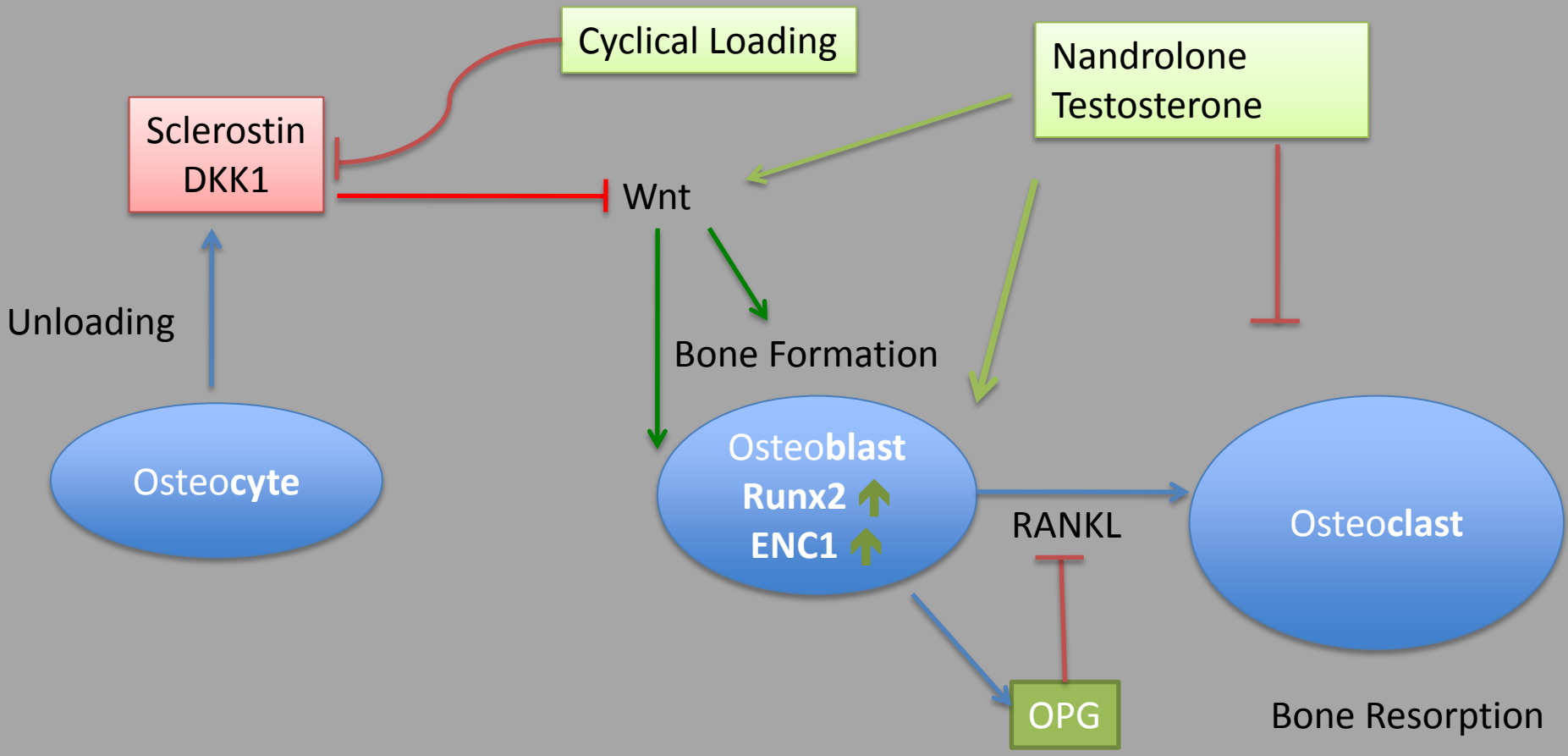
Nandrolone Increased OPG Expression



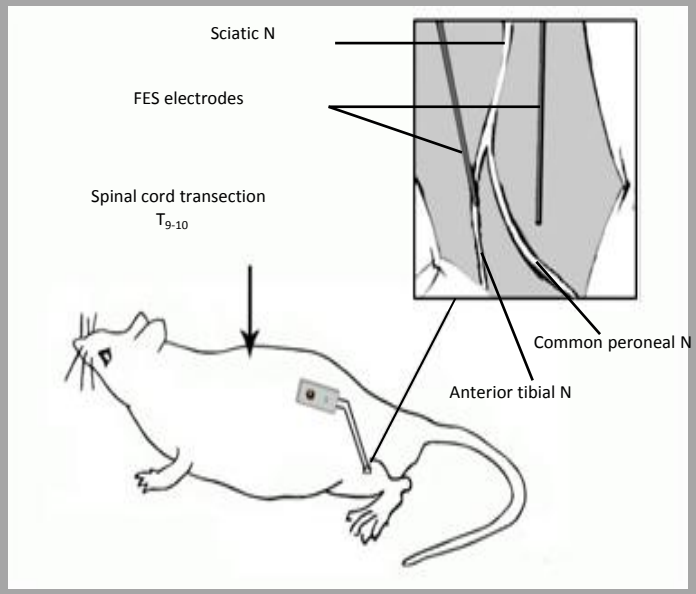
Nandrolone Increased Wnt Signaling Genes

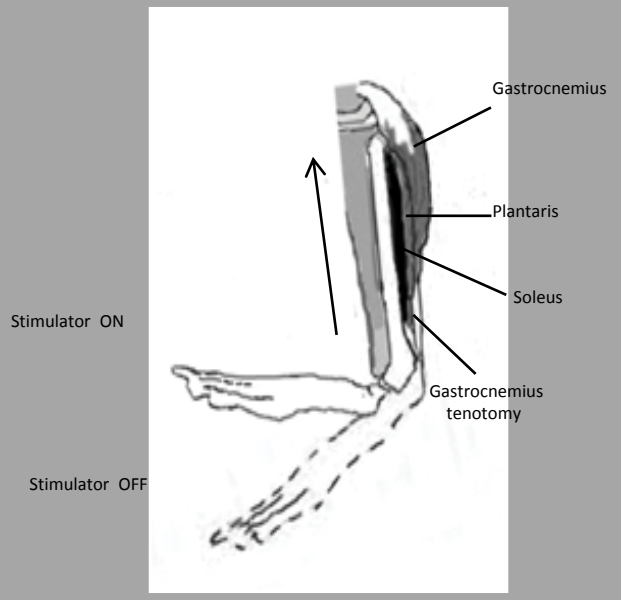


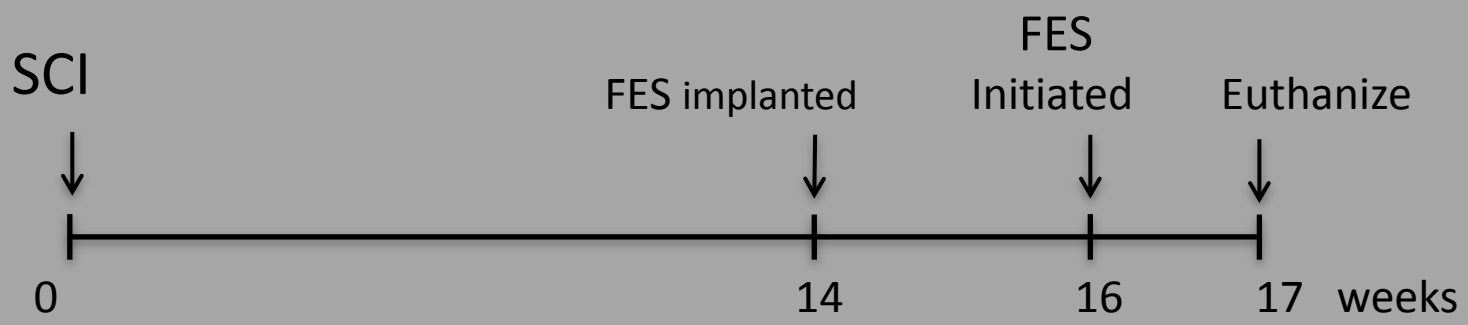
Key Molecular Signals in Bone



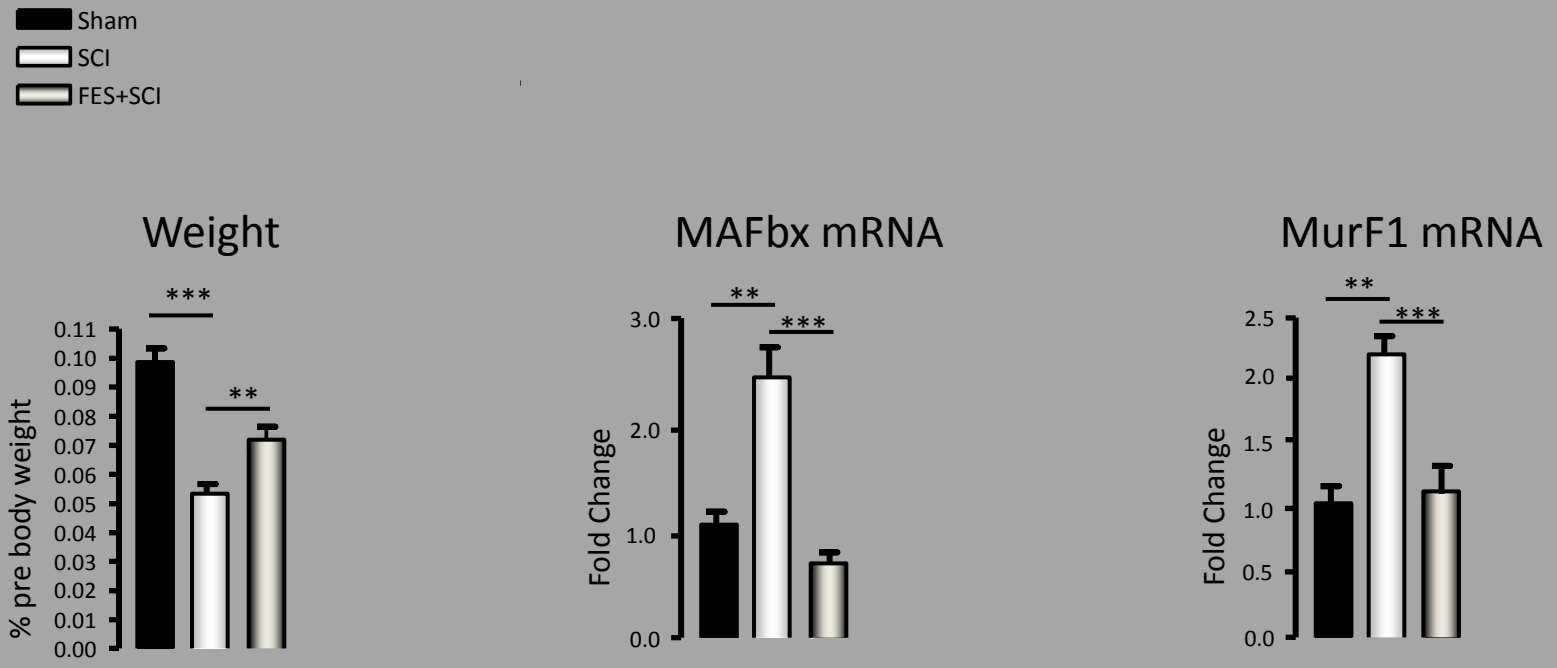
FES&Bone: Experimental Design





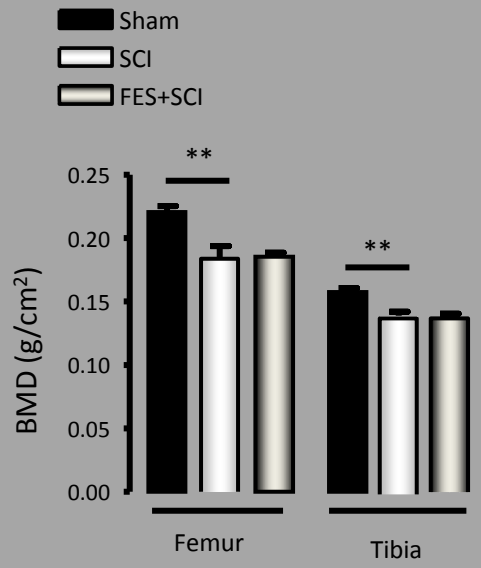


Effects on the Plantaris Muscle of FES for 7 Days

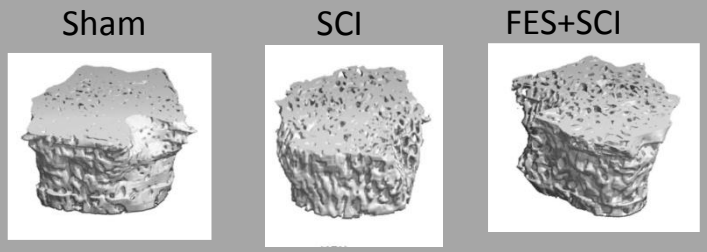


SCI Led to Reductions in Bone Mass

Areal BMD by DEXA Scan:
Distal Femur and Prox. Tibia

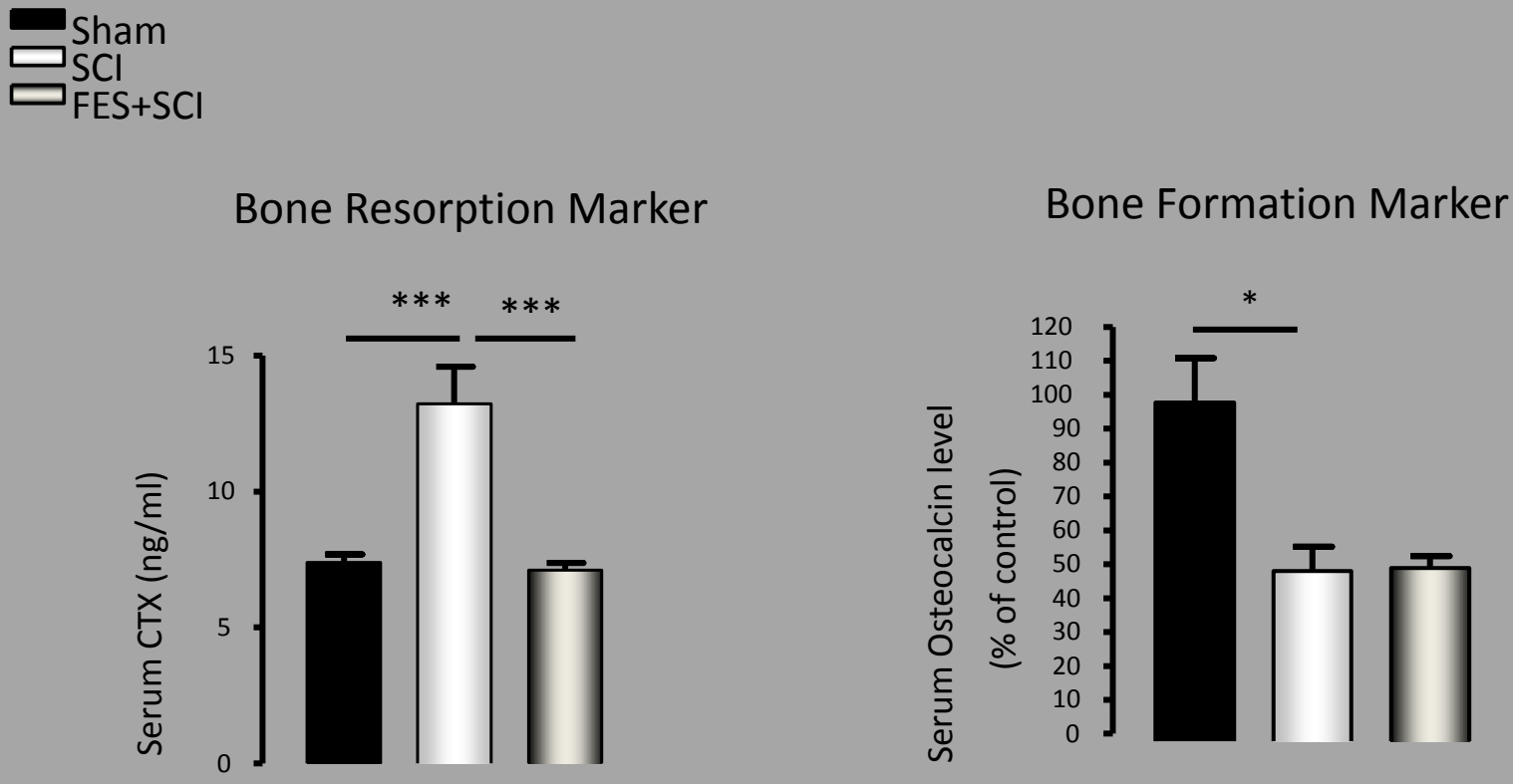


MicroCT Studies: Prox. Tibia



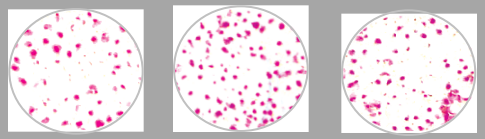
	Sham	SCI	SCI+FES
BV/TV(%)	0.65±0.04	0.34±0.01 ***	0.30±0.02 NS
Tb.N (μm^{-1})	6.09±0.32	4.77±0.05 ***	4.42±0.09 NS
Tb.Th (μm)	0.13±0.01	0.09±0.02 **	0.08±0.01 NS
Tb.Sp (μm)	0.11±0.01	0.18±0.01 ***	0.19±0.01 NS

Effect of FES on Blood Markers of Bone Metabolism



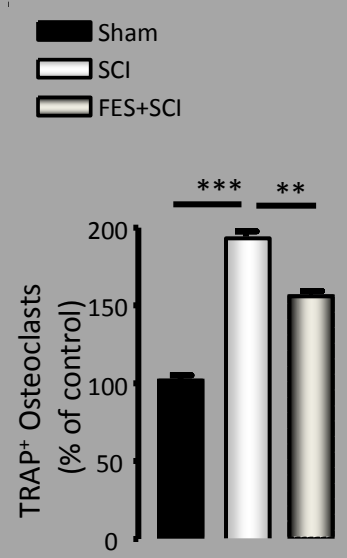
Properties of Ex-vivo Cultures of Osteoclasts

TRAP staining

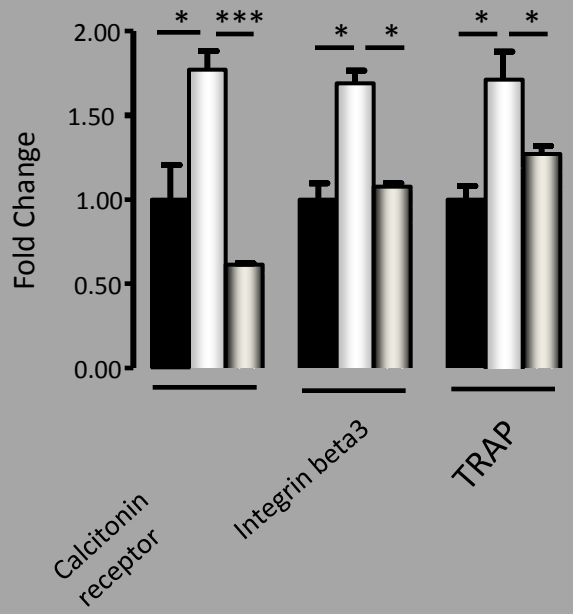


Sham SCI FES+SCI

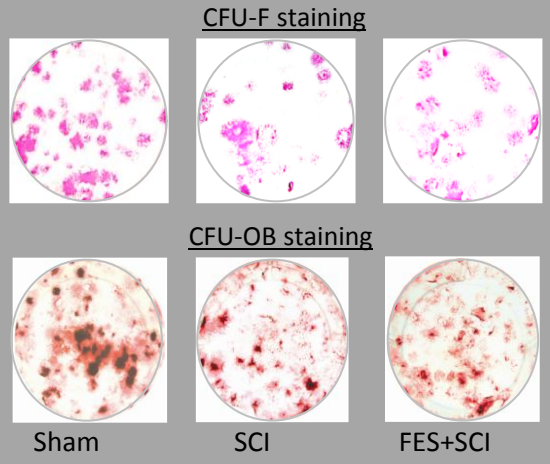
Osteoclast Counts

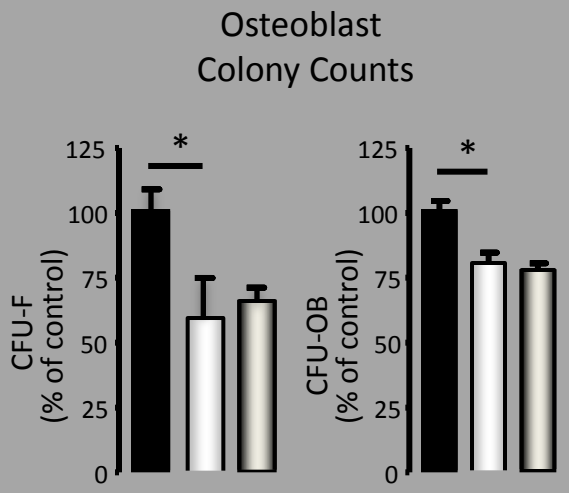


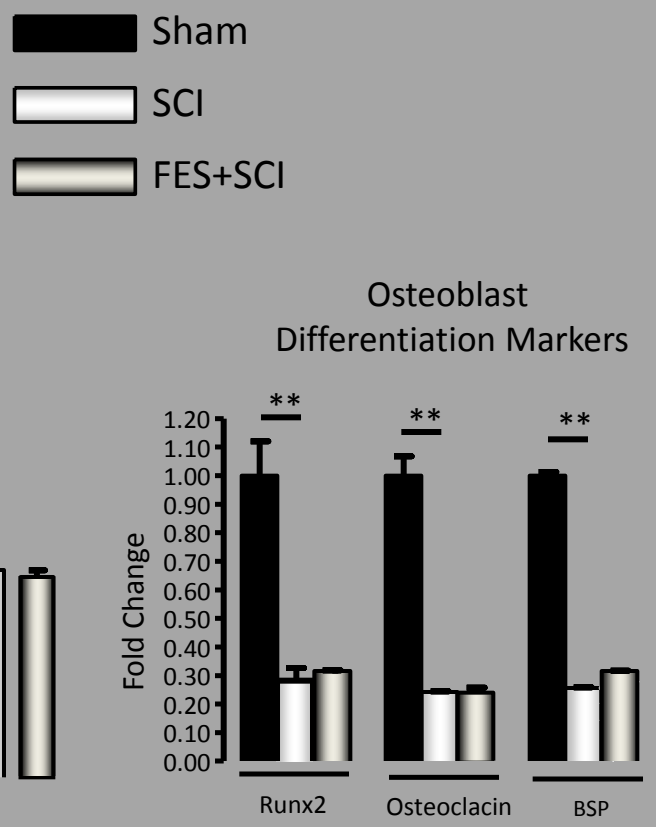
OC Differentiation Markers



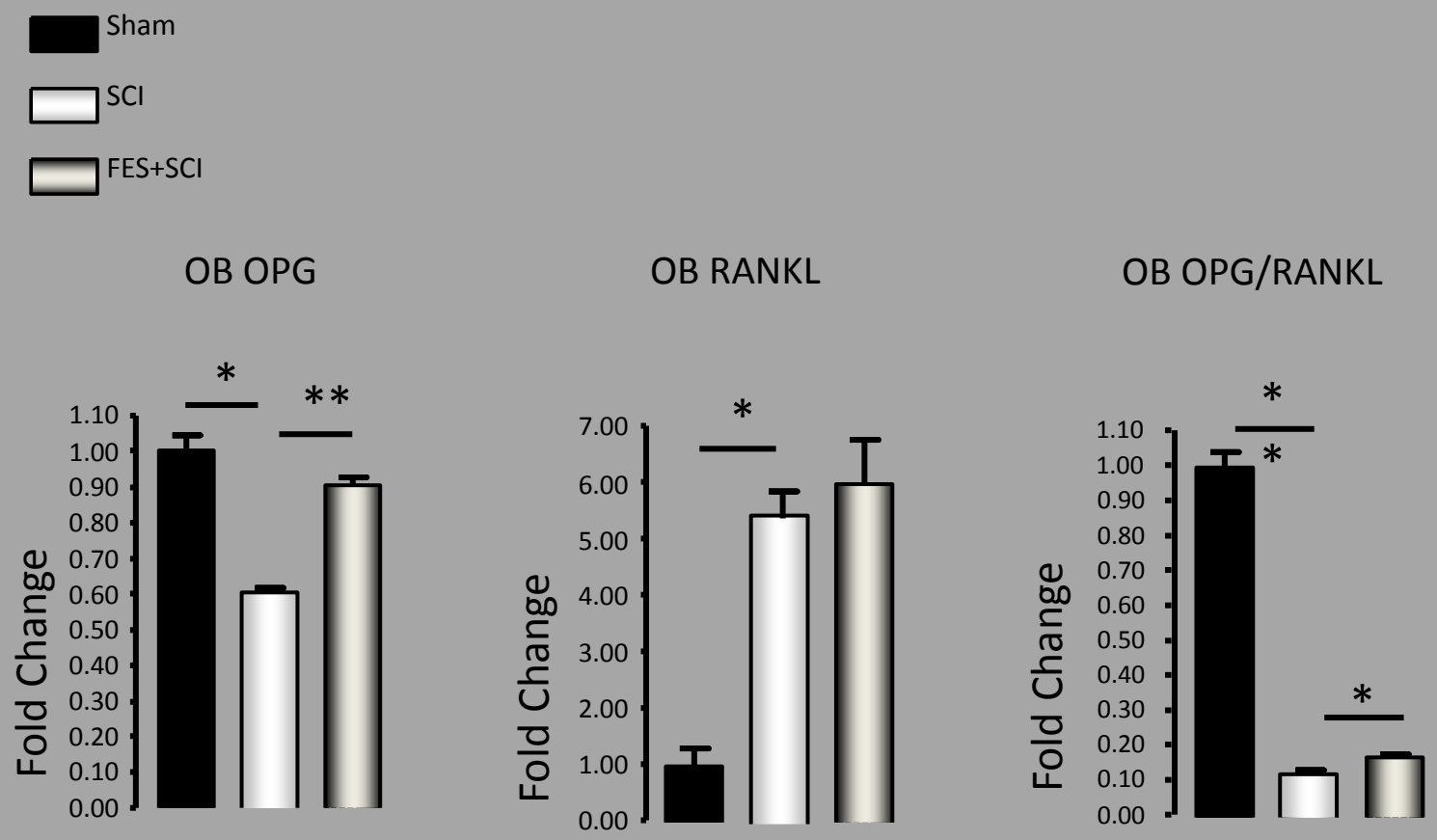
Properties of Ex-vivo Cultured Osteoblasts



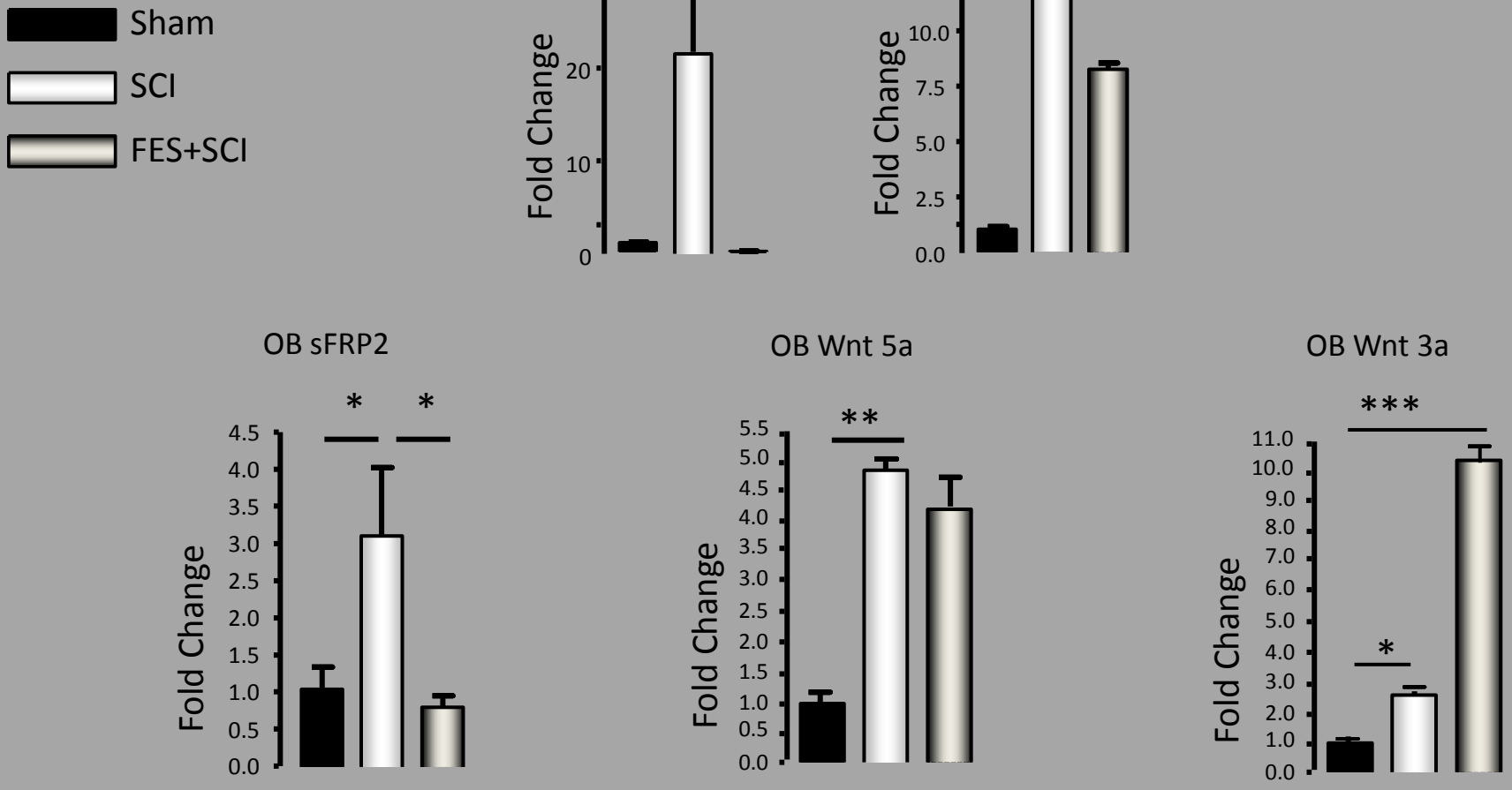




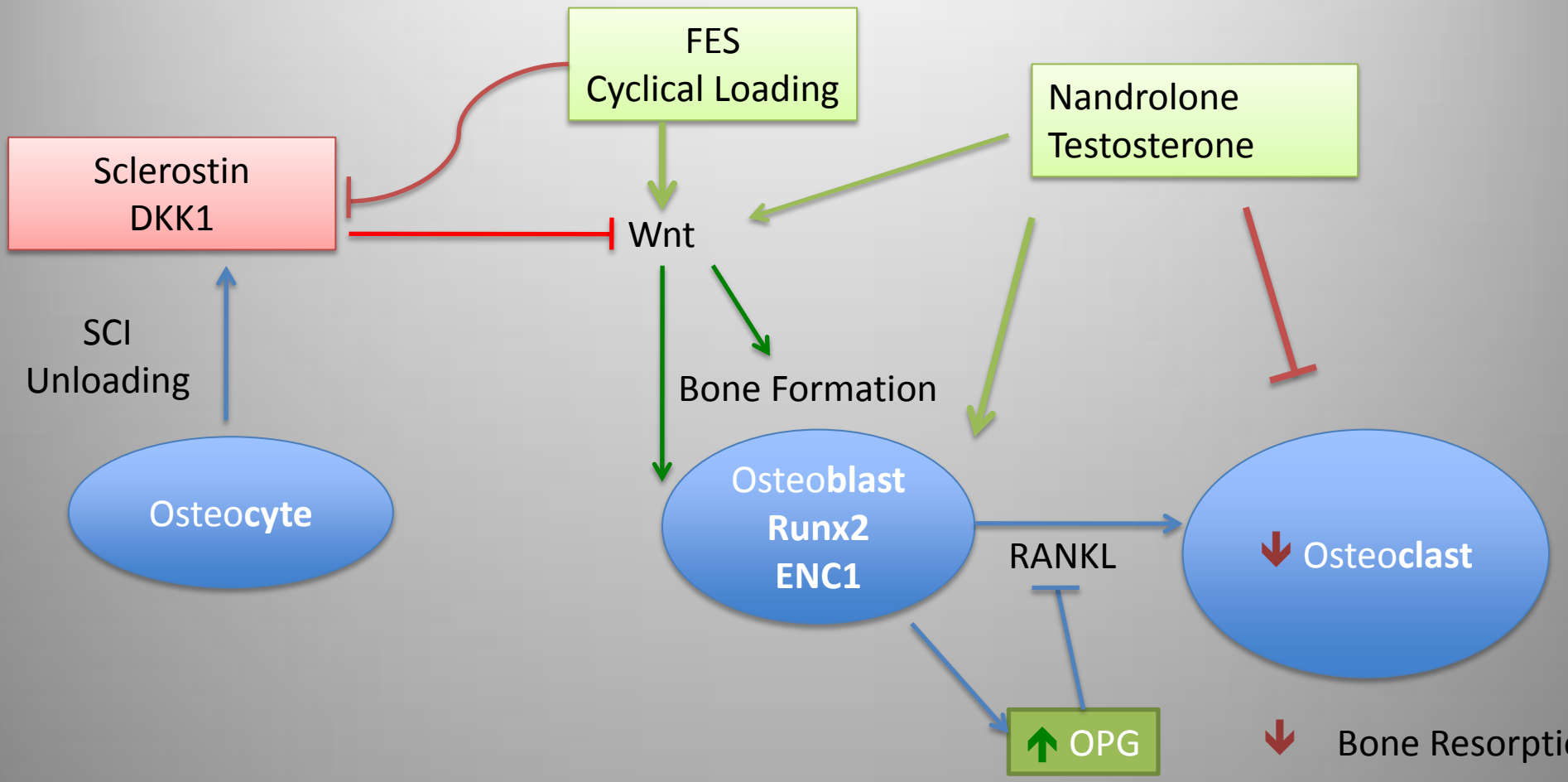
Expression in Ex-vivo Cultures of Osteoblasts (OB) of RANKL and OPG



Expression in Osteoblasts of Wnt-Signaling Molecules



Key Molecular Signals in Bone



Microarray Analysis of Gene Expression in Osteoblasts and Osteoclasts

Table 1. Selected results of a pathways analysis of the microarray data.

Osteoblasts		Osteoclasts	
Hedgehog-PTH signaling	15/37 genes, p 8.1×10^{-7}	PTH Signaling, Vitamin D Receptor Signaling	9/37 genes, p 1.62×10^{-2}
ß-Adrenergic signaling	12/52 genes, p 3.85×10^{-3}	Oxytocin Signaling	12/59 genes, p 1.57×10^{-3}
Wnt Signaling	12/53 genes, p 4.55×10^{-3}	FSH Signaling	9/40 genes, p 2.89×10^{-3}
		Calcium/NFAT Signaling	9/40 genes, p 2.89×10^{-3}
			10/57 genes, p 1.11×10^{-2}

Early Effects of Reloading by FES on Bone

- In a subacute model of SCI:
  - FES rapidly reduces the accelerated bone resorption that is a characteristic of SCI.
  - FES reduces expression of inhibitors of Wnt signaling (SOST, DKK1 and sFRP2).
  - FES increases expression of Wnts and the Wnt-responsive gene OPG.
  - Increased OPG explains in part the favorable effects of FES on bone resorption after SCI.

What Clinical Directions do Studies in Animal Models of SCI Support

- Androgens may be beneficial to bone and may reduce bone resorption to a clinically meaningful degree.
- Interventions targeted against Wnt inhibitors, such as sclerostin may be beneficial after SCI.
- Interventions targeting RANKL may reduce bone resorption during the subacute period after SCI.
- Animal models permit the study of acute and subacute periods after SCI.
- There is a lack of animal systems that model the chronic phase of SCI-related bone loss.

- James J. Peters VAMC
  - Yong Wu
  - **Weiping Qin**
  - Yiwen Qin
  - **Lauren Collier**
  - Jiangping Pan
  - Xin-hua Liu
  - **Bill Bauman**
- MSSM
  - **Mone Zaidi**
- Cleveland ATP COE
  - **Graham Creasy**
- University of Liverpool

  Jonathan Jarvis

Obtaining CME Credit

- If you would like to receive CME credit for this activity, please visit:

  http://www.pesgce.com/PVAsummit2011/

- This information can also be found in the Summit 2011 Program on page 8.