



CENTER FOR DISRUPTIVE
MUSCULOSKELETAL INNOVATIONS

***Integrated in vivo and in vitro
high-throughput analyses of
osteocyte-mediated bone remodeling***

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WWW.NSFCDMI.ORG

Project Aims

This project aims to develop a comprehensive approach to evaluate periacicular remodeling (PLR) in vivo and in vitro to advance the development of diagnostics and therapies to improve bone quality.

Aim 1: Develop and validate in vitro measures of PLR function for high throughput screening.

- currently, there is no validated in vitro PLR assay

Aim 2: Establish the Osteocyte-Mediated Bone Remodeling ECM (OMBRE) Core.

OMBRE Protocols I-V 1

I: Collagen Organization

II: Lacunocanalicular Analysis

III: PLR Gene Expression

IV: In Vitro Functional pH Assay

V: In Vitro PLR Reporter Assay

2
High Throughput Screen

3
CDMI Access to OMBRE Services through the UCSF Skeletal Biology and Biomechanics Core

OMBRE Core Services

UCSF Skeletal Biology
Core Director:
Tamara Alliston

1 **Progress Update**

Updated website:
[CCMBM](#)

2 **Progress Update**

3 service requests
(UC Davis, UCSD,
Orthofix)

3 **Progress Update**

OMBRE core
protocols available
on [Airtable](#)

4 **Progress Update**

developed "OMBRE"
text for grants and
project strategies

CDMI

Skeletal Biology Core Services



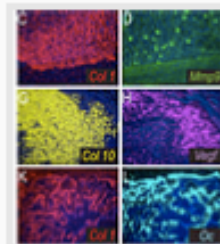
Imaging and Histology Sub-Core

Provides imaging services for small animals and tissue specimens using computed X-ray tomography. Offers technical support in tissue extraction and processing for histology, as well as in histomorphometric analysis.



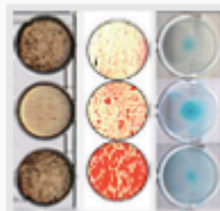
Biomechanics Sub-Core

Offers resources and expertise in quantitatively evaluating the mechanical and material properties of skeletal tissue over a range of resolutions and scales.



Molecular Biology Sub-Core

Provides expertise in the isolation of RNA and protein from skeletal tissue, the quantitative analysis of gene and protein expression using real-time qPCR and Western blotting, and the qualitative analysis of gene and protein expression through in situ hybridization and immunohistochemistry.



Cell Culture Sub-Core

Maintains and supplies a collection of chondrocytic, osteoblastic, and osteoclastic cell lines and provides expertise in preparing primary cultures of murine bone marrow stromal cells and other cell populations. Offers technical support in the use of stains and enzymatic assays to assess bone-related properties in cultures.

Click [HERE](#) for a list of currently available cell lines.

OMBRE Protocols I-V

I: Collagen Organization

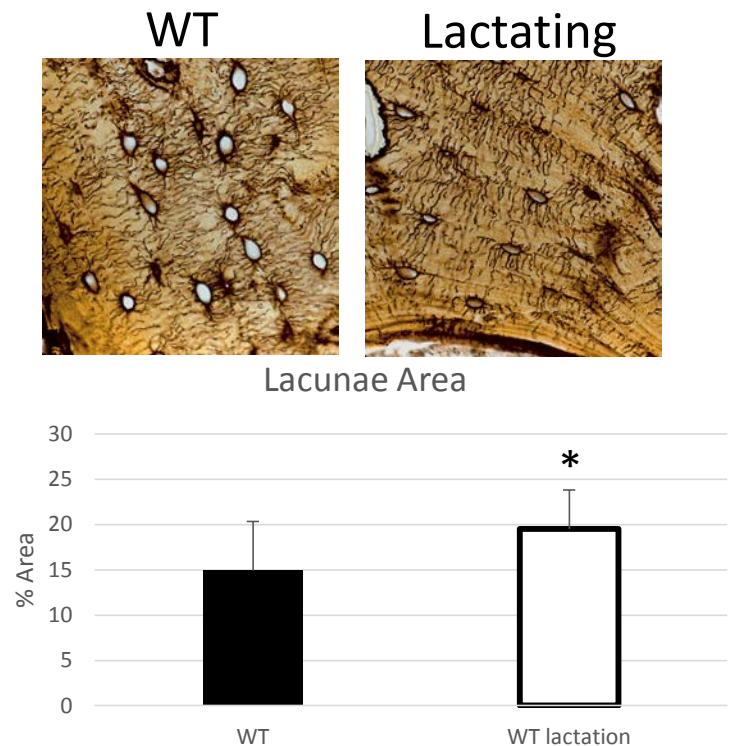
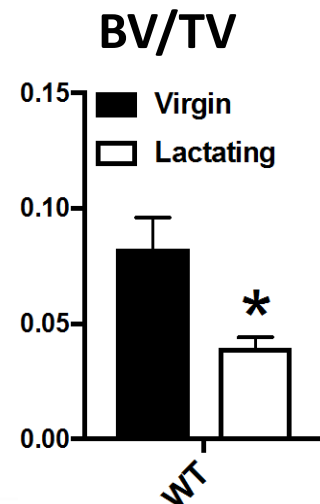
II: Lacunocanalicular Analysis

III: PLR Gene Expression

IV: In Vitro Functional pH Assay

V: In Vitro PLR Reporter Assay

- 5** Progress Update
- Harvested tissues for OMBRE validation
- virgin and lactating mice
 - microCT validation complete
 - histology and quantification on lacunocanalicular complete



OMBRE Protocols I-V

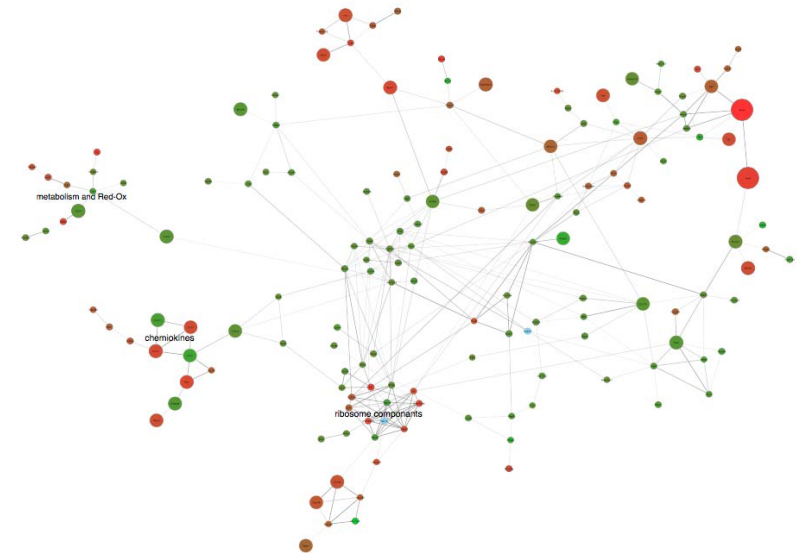
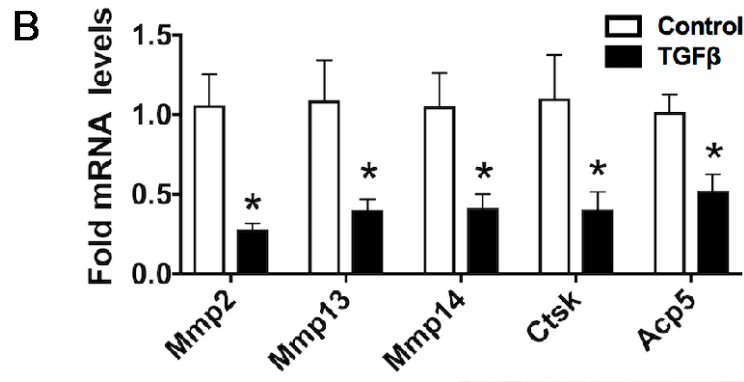
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Progress Update

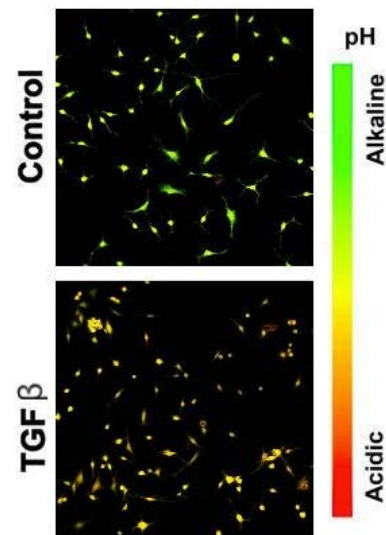
Harvested tissues for OMBRE validation

- PLR Gene Expression down regulated in $TBR1^{ocy-/-}$ mice
- Analysis of RNAseq is complete with 369 genes
- 10 genes of interest are for upcoming validation



OMBRE Protocols I-V

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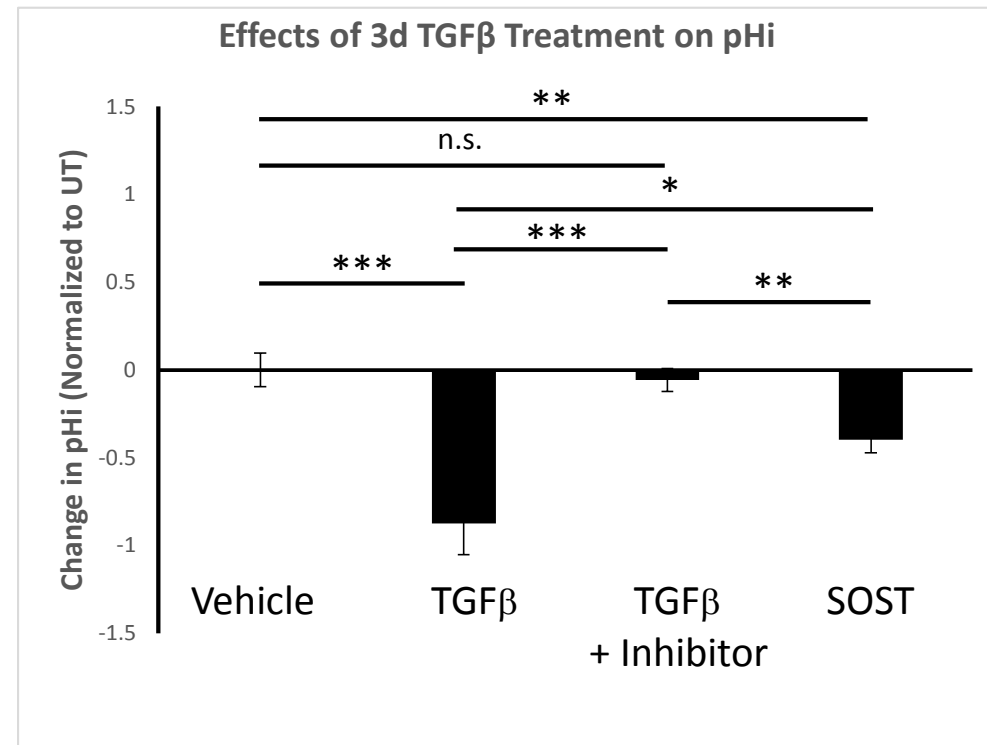


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Progress Update

Developed & validated in vitro pH assay

- pH regulation matches PLR regulation
- performing viability assay in parallel with pH assay



Milestones

In Vitro Assays

- Identify prototypical PLR-inducible gene for 1st in vitro PLR functional outcome
 - Dec 1, 2016
 - **STATUS: RNAseq data analyzes complete**
- Finish cloning and sequence validation of novel PLR-report construct
 - April 1, 2017
 - **STATUS: validation of RNA seq hits is underway (10 genes of interest)**
- Finish development of 2nd in vitro PLR functional outcome: intracellular pH assay
 - February 1, 2017
 - **STATUS: Protocol complete & protocol written, cell viability is underway**

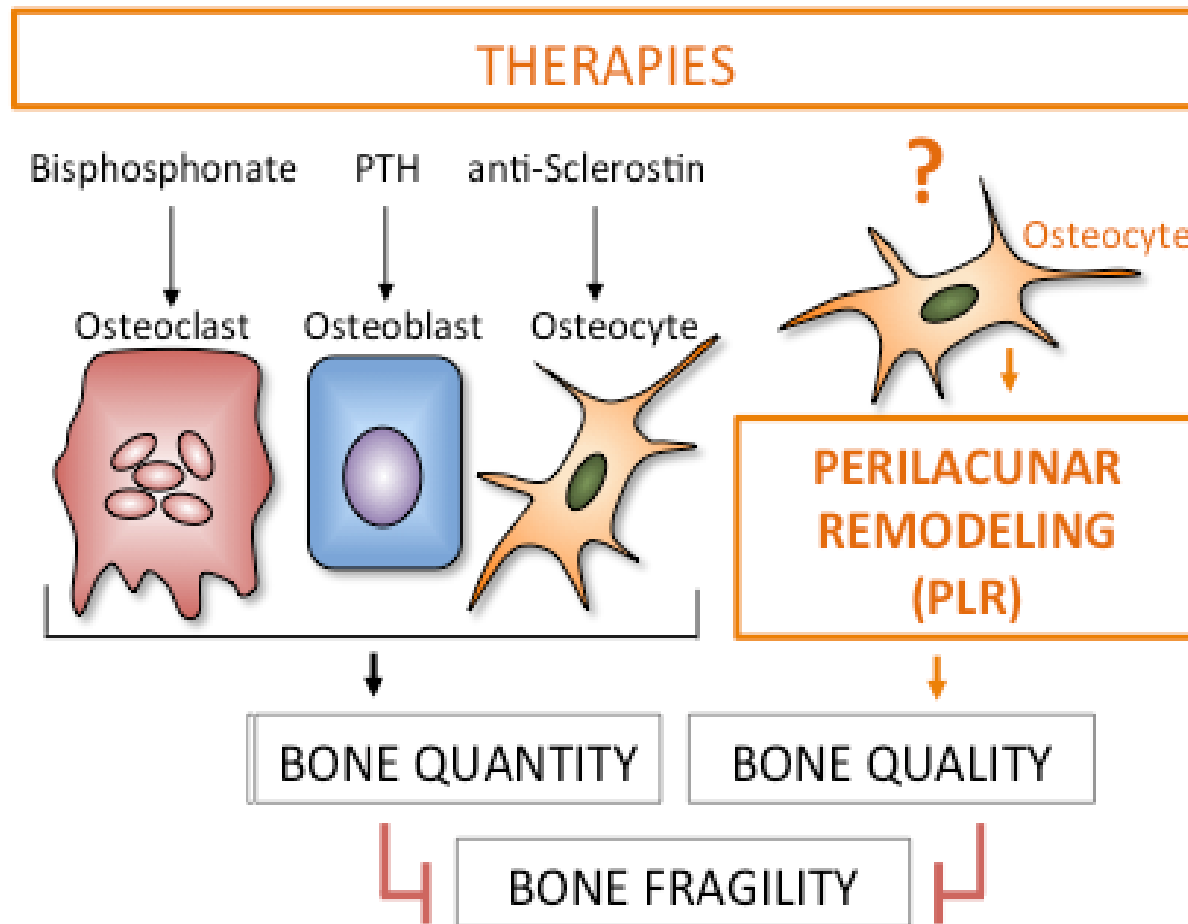
In Vivo Assays

- Final protocol for OMBRE I: Collagen Organization
 - January 15, 2017
 - **STATUS: Protocol complete, validated in lactating mouse bone**
- Final protocol for OMBRE II: Canalicular Stain
 - March 1, 2017
 - **STATUS: Protocol complete, quantitative analysis complete**
- Finish protocol for OMBRE III: PLR Gene Expression
 - May 1, 2017
 - **STATUS: Protocol complete, validated in TBR1^{ocy}^{-/-} mice**

Clinical Need and Industrial Relevance

Contributors

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Steroid Use
Osteonecrosis
Osteoarthritis
Aging
Diabetes