Functional Analysis of *SIX1* in Osteoblast Activity

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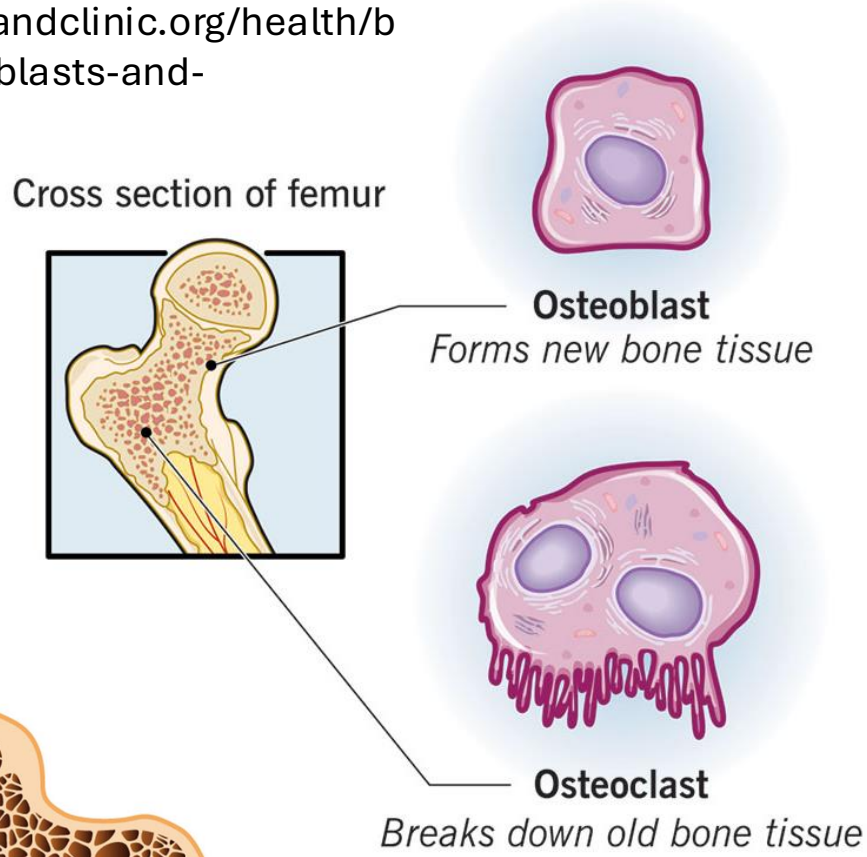
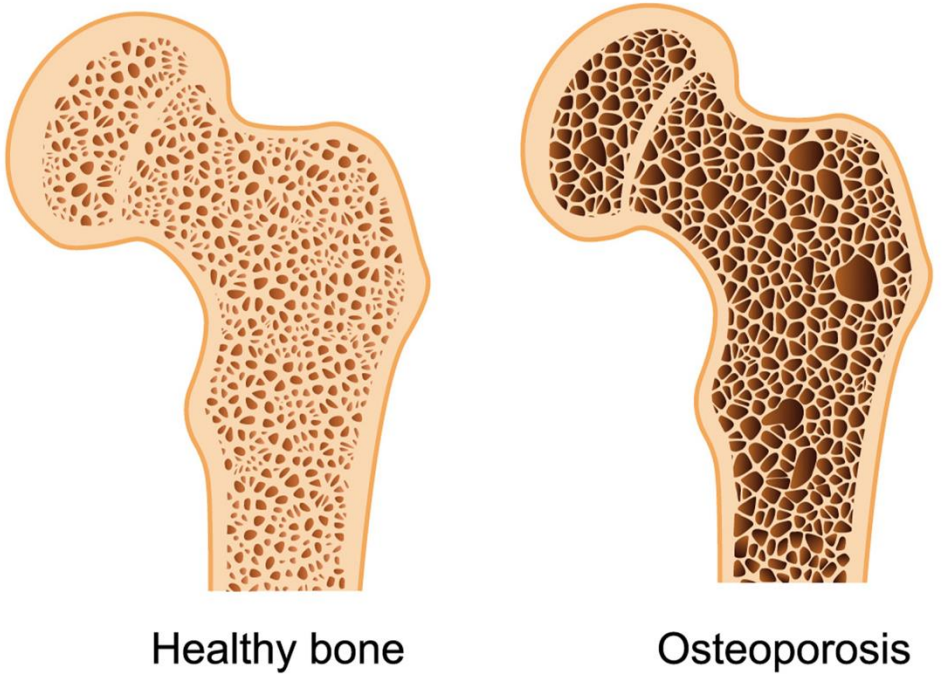
Osteoblasts

https://my.clevelandclinic.org/health/body/24871-osteoblasts-and-osteoclasts

- Regulate bone formation and mineralization
- Dysregulation leads to diseases like **osteoporosis**, marked by low bone mineral density (BMD)
- One BMD genetic regulator of interest is *SIX1*



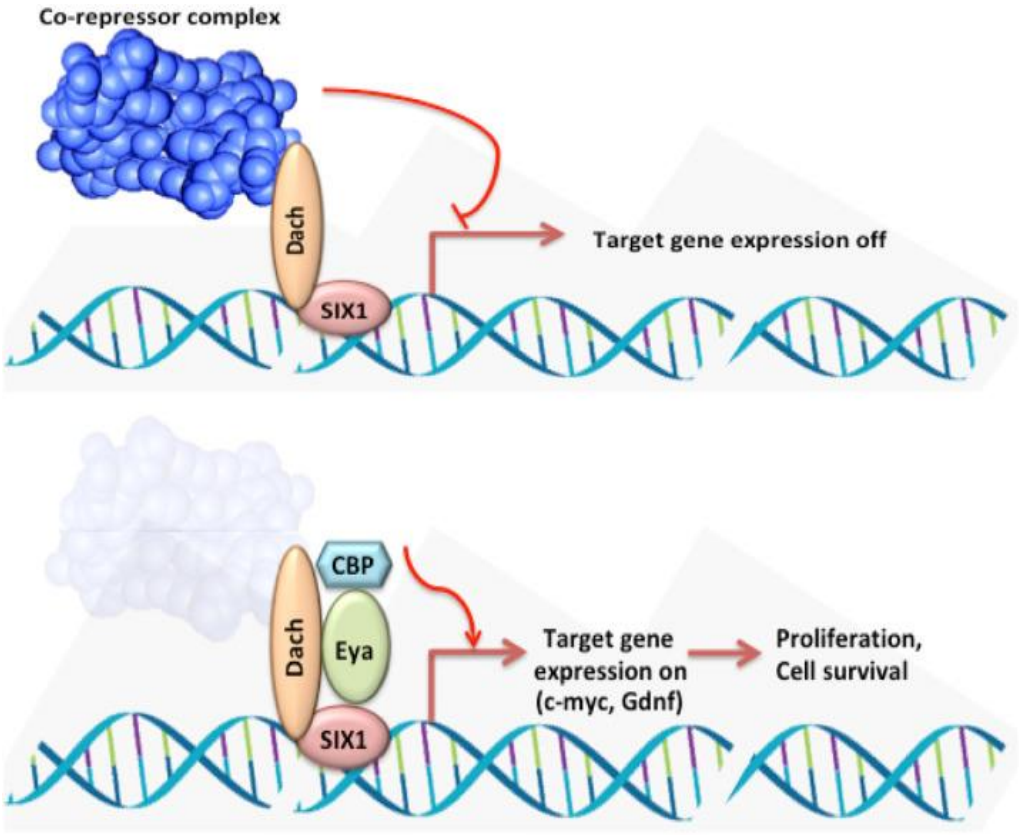


https://www.desertspineandsports.com/osteoporosis-and-bone-health/

SIX1

- Sine Oculis Homeobox 1
- From *SIX1–SIX6* family of genes
- Transcription factor during embryonic development
- Regulates De novo lipogenesis
- Mechanism is poorly understood in bone

  → Does *SIX1* influence osteoblast cell maturation via omega-3 fatty acid production?



https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/six1

Does reduced expression of *SIX1* impact osteoblast activity?

Objectives

- Generate human fetal osteoblasts (hFOBs) *SIX1* knockdown cells (hFOB $SIX1^{kd}$)
- Investigate the role of *SIX1* in hFOBs
- Predict other osteoporosis-related genes or genes that interact with *SIX1*

Transfection with siRNA *SIX1*

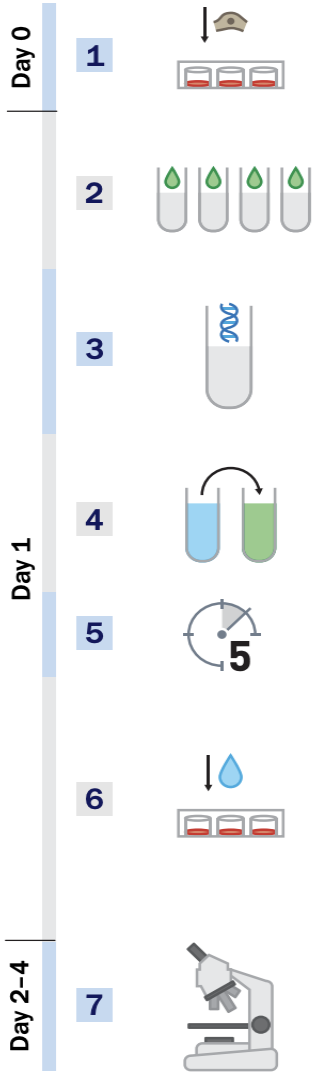
Seeded 250,000 hFOB cells in 6 wells

- ~90% confluency

Transfection with siRNA to obtain *SIX1*^{kd}

- Lipofectamine 2000
- Scramble control (*B2M1*) vs. knockdown (kd)
- Biological triplicates

Cellometer K2 Fluorescent Cell Counter and Brightfield microscope



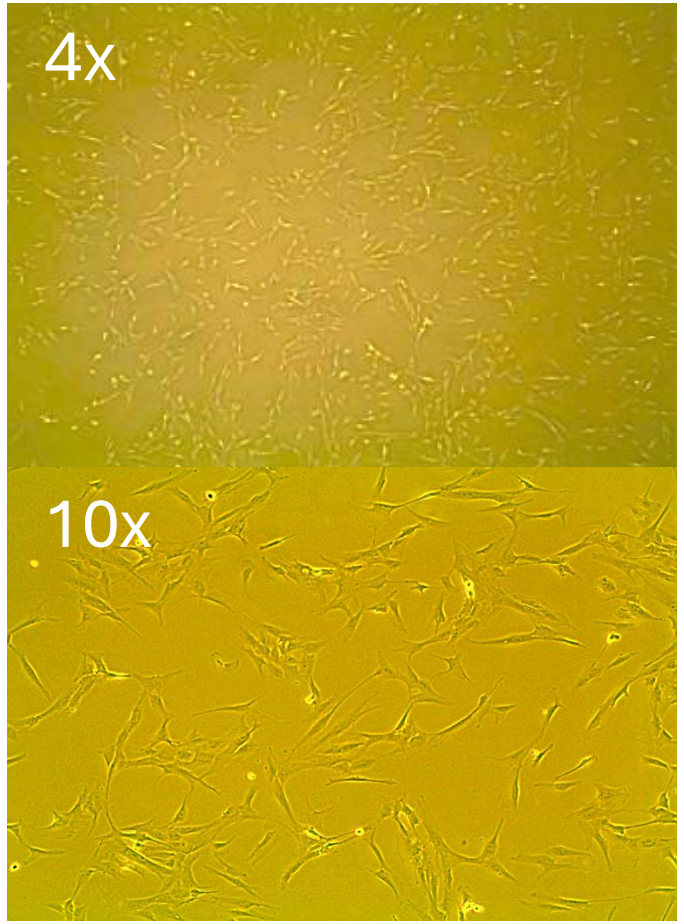


Figure 1. Brightfield images of hFOBs at Passage 2 under (A) 4× and (B) 10× magnification. Cells show typical healthy morphology during the proliferative phase. Images were taken prior to transfection to confirm adherence and growth.

SIX1 Gene Expression

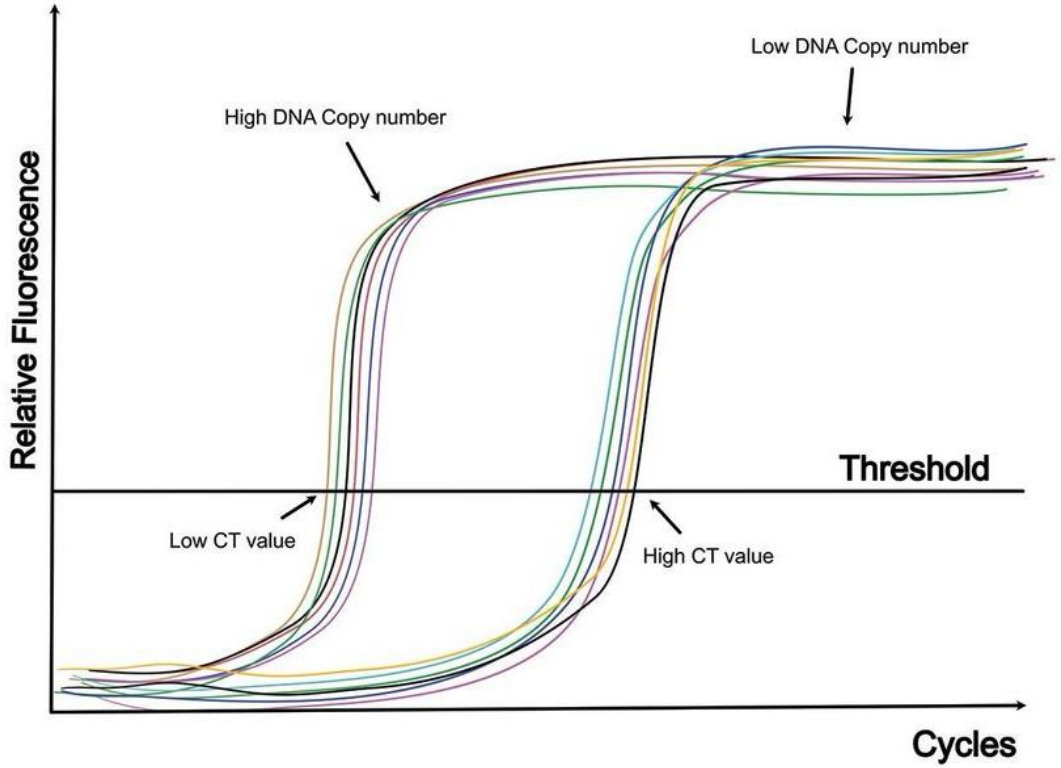
Quantitative Polymerase Chain Reaction (qPCR) 24 hours post transfection

- Amplified scramble control hFOB cells and hFOB^{kd} cells
- Gene expression = mRNA levels
- Converted mRNA to complimentary DNA (cDNA) prior

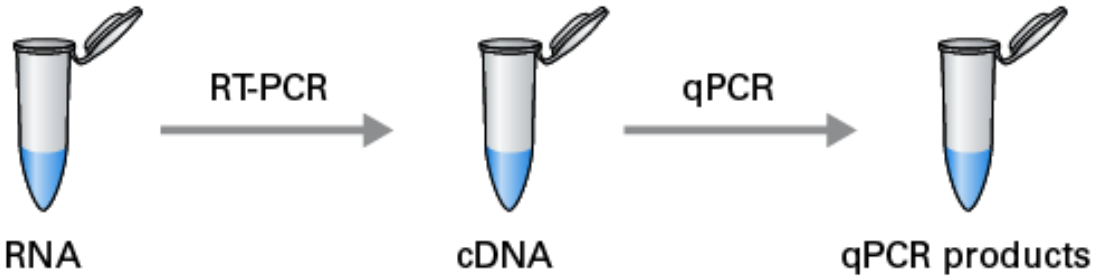
Fold change (FC) calculation

- Higher cycle threshold (CT) = lower *SIX1* expression
- Lower CT = higher SIX1 expression
- FC= $2^{-\Delta\Delta CT}$

https://www.the-scientist.com/insights-into-qpcr-protocol-detection-methods-and-analysis-71478



https://www.takarabio.com/about/bioview-blog/tips-and-troubleshooting/one-step-vs-two-step-rt-qpcr



Results

Findings: Fold change was nearly unchanged.

siRNA knockdown of *SIX1* was not effective

- Incorrect primers
- siRNA not potent enough
- Knockdown is time dependent
  - qPCR after 48 or 96 hours

→ Does reduced expression of *SIX1* impact osteoblast activity?

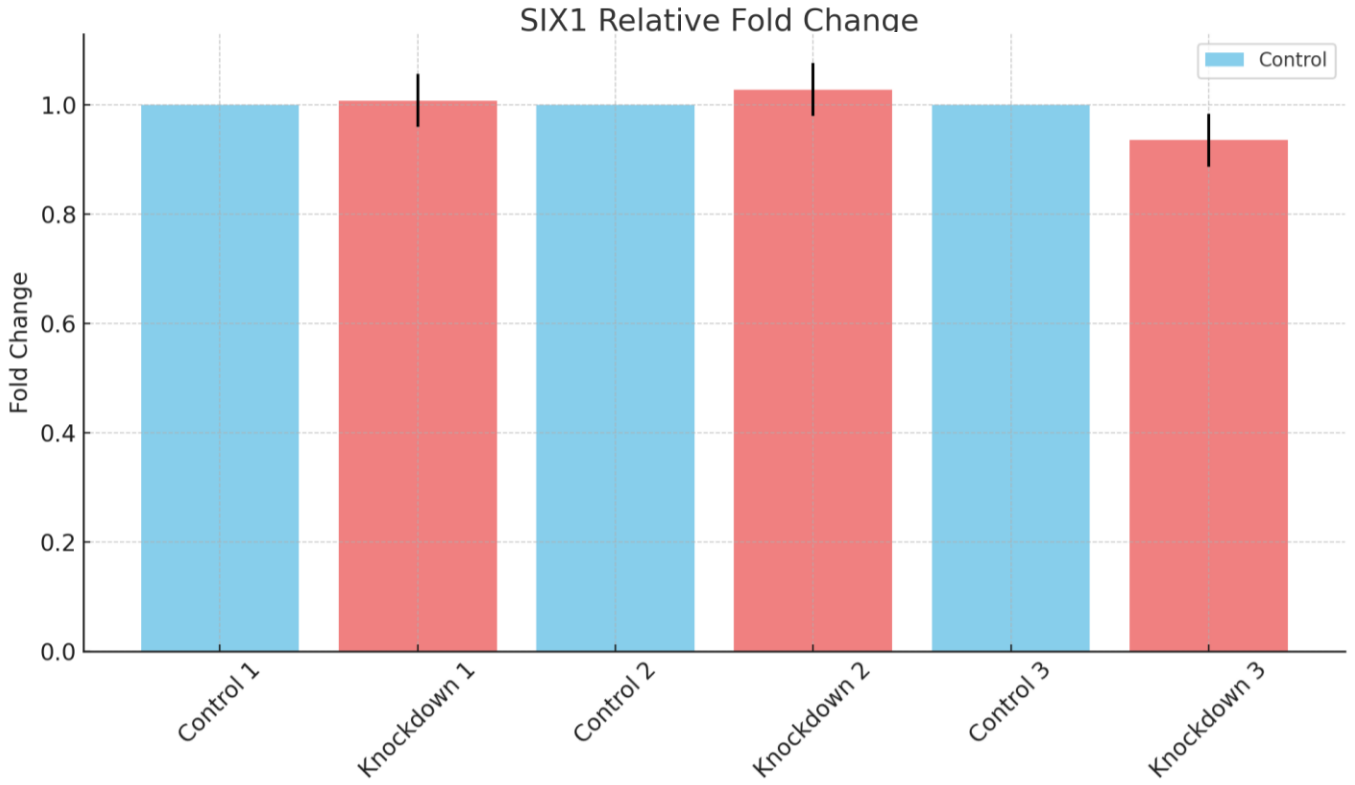
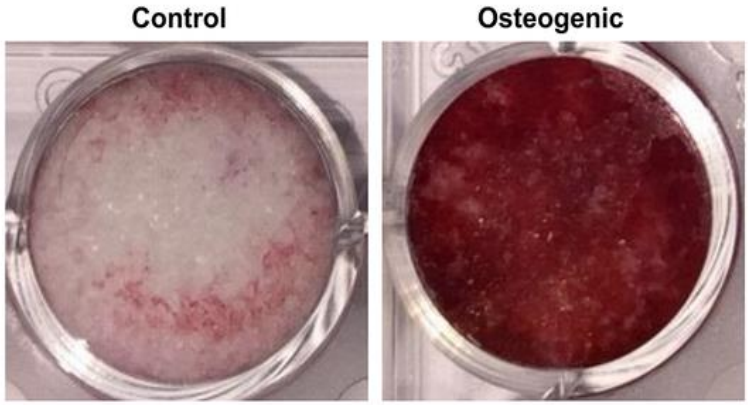


Figure 2. qPCR analysis of SIX1 mRNA expression in hFOB cells 24 hours after siRNA transfection. Fold change in *SIX1* expression was normalized to the housekeeping gene *B2M1*. No significant reduction in SIX1 expression was observed in knockdown samples compared to controls ($p > 0.05$, unpaired T-tests), indicating unsuccessful gene silencing at 24 hours.

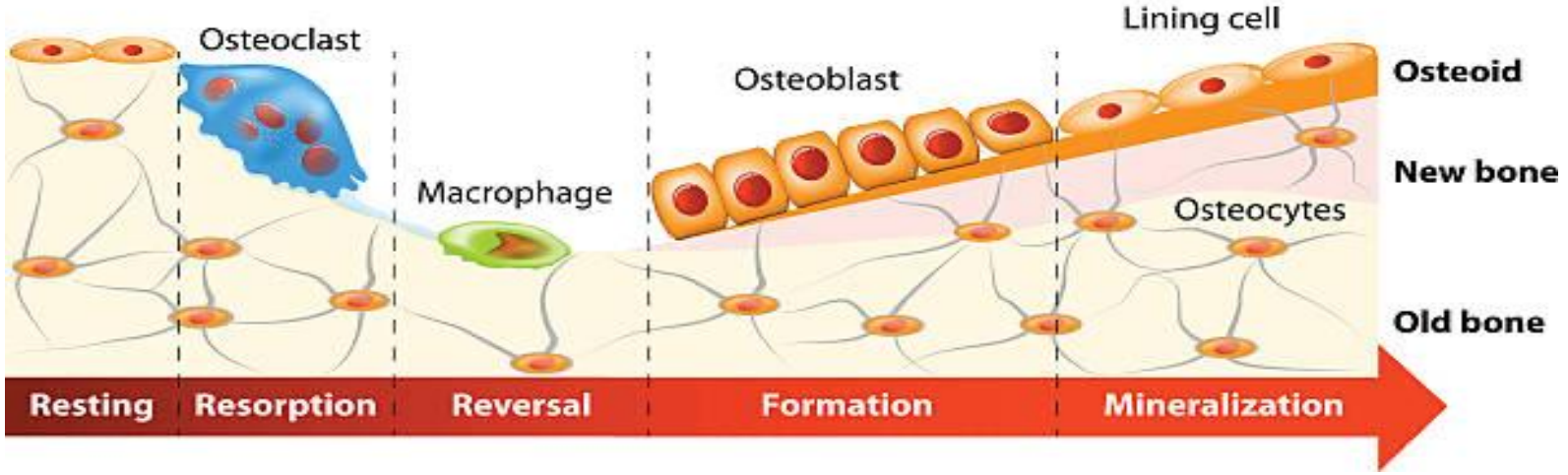
Future Direction



https://www.researchgate.net/figure/Mineralization-assay-of-cBM-MSCs-seeded-PCL-HA-constructs-A-Schematic-experiment-of_fig5_383878741

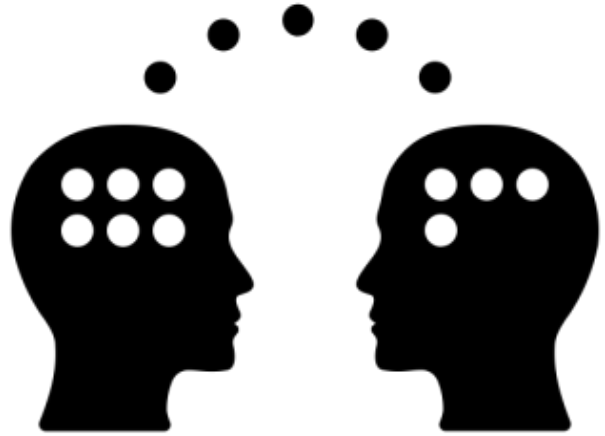
Examine phenotypic changes in hFOB cells

- qPCR and Western blotting
- Measure osteoblast activity:
  - → Proliferation Assay
  - → Migration Assay
  - → Mineralization Assay



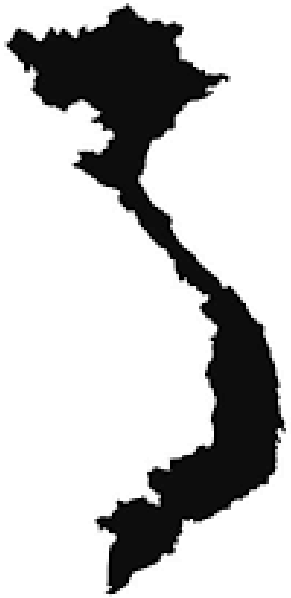
https://www.istockphoto.com/vector/bone-remodelling-process-gm494890194-77697629

Cultural Exchange



- Coffee!
- Vietnamese and Indian food
- Upbringing and schooling
- Weekly Journal Meetings
  - Friday presentations from Dr. Ackert-Bicknell's team
  - Played to speaker's background and strengths, exploring:
    - → Osteoclasts and bone remodeling
    - → ANOVA statistics
    - → Coding

https://www.siftandsimmer.com/ca-phe-sua-da-vietnamese-iced-coffee/



Thank you!

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