## Single-Cell RNA Sequencing of Repair Tissue Formed After Growth Plate Injury Reveals a Potential Role for Macrophages and Mesenchymal Progenitor Cells in Bony Bar Formation

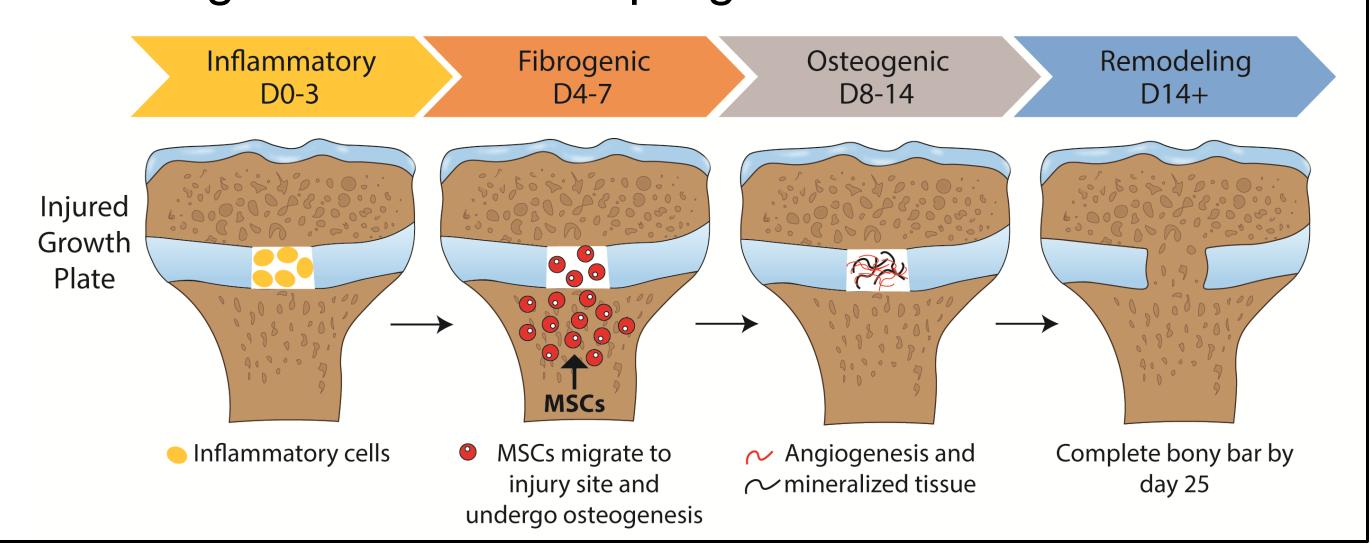
Aditya Mantha, Stacey M. Thomas, MS; Ethan J. Beltrand; Karin A. Payne, PhD

Department of Orthopedics, Colorado Program for Musculoskeletal Research, University of Colorado Anschutz Medical Campus



## BACKGROUND

- Growth plate is a region of cartilaginous tissue; when injured, it can be replaced with bone tissue (bony bar)
- Driven by various inflammatory and osteogenic processes
- Purpose: Identify MSC populations in the injured site at different time points following a growth plate injury and detect genes that were upregulated at these times



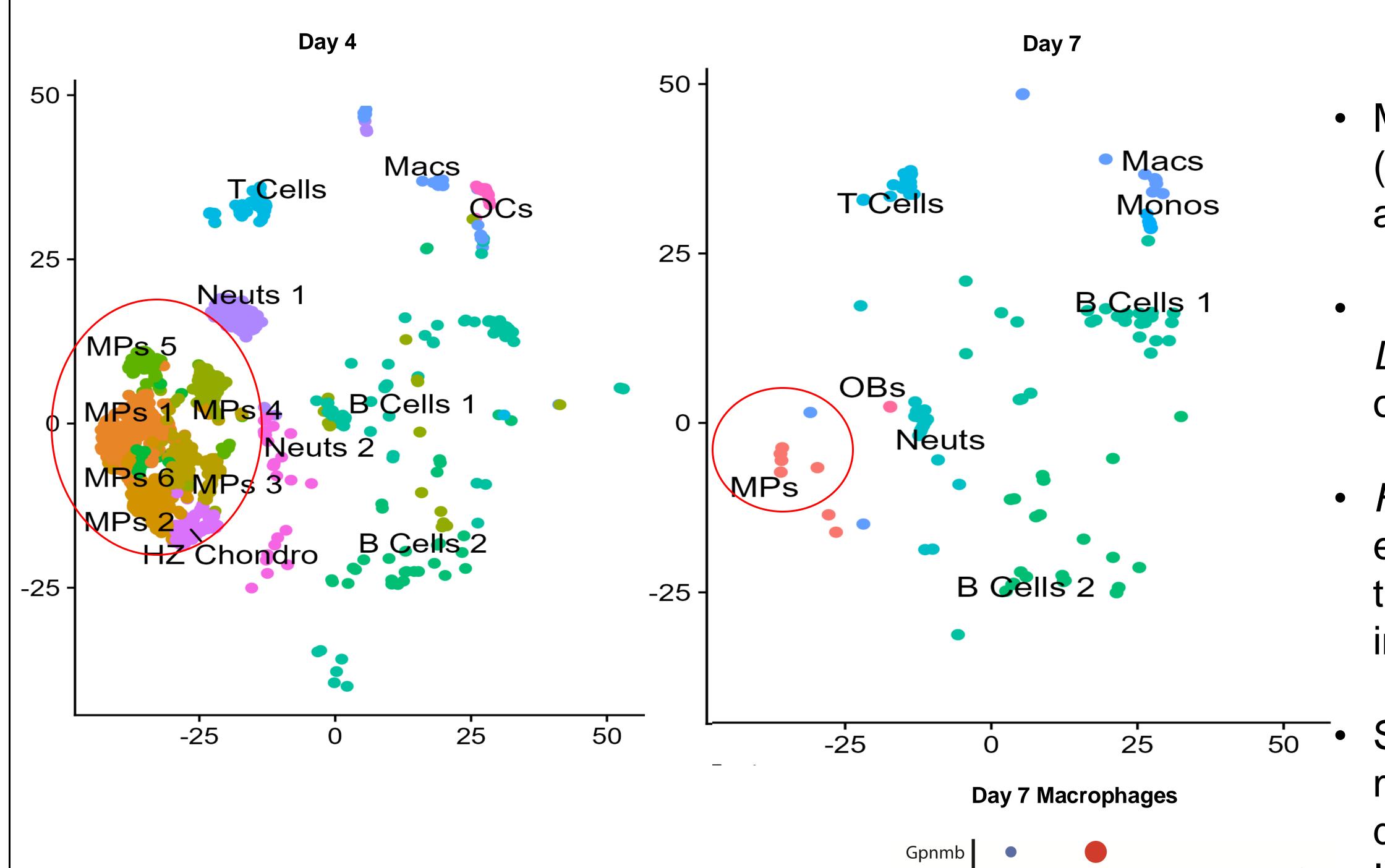
## A and 7 days post-injury (n=2/timepoint) 2 Dissection and digestion of repair tissue 3 Single-cell sorting by FACS Cell type 1 Cell type 1 Cell type N

5 Single-cell expression profile

6 Clustering & cell type identification

4) Single-cell sequencing

## RESULTS & DISCUSSION



Percent Expressed Osm Lepr Percent Expr 80100 Col1a1 • 50 Tgfb1 **•** 75 **Average Expression** Bglap Pdgfa Mrc 1 Average Exp<sub>Mmp 14</sub> Ibsp 2.25 2.00 Spp1 Vcan 1.75 Vegfa Runx2 1.50 Nos2 1.25 1.00 Alp Ptgs2 Bgn Tnf Cd69 MPs 2 MPs 3 MPs 4 MPs 5 MPs 6 MPs Cluster Cluster Day 7 Day 4

- Mesenchymal progenitor cells (MPs) were identified at days 4 and 7 after growth plate injury
- Ogn, Bgn, Alpl, Runx2, and Lmna are genes that promote osteogenesis
- Foxa2 (not pictured) was expressed in MP on Day 4 and the which has been implicated in chondrogenesis
- Sub-clustering of the day 7 macrophages uncovered two distinct clusters: Pro-line
   Inflammatory and Pro-Reparative
  - Pro-reparative macrophages expressed *Gpnmb*, which promotes MSC migration, proliferation, and survival
  - Pro-Inflammatory macrophages expressed COX2, iNOS, and Vegfa