# Joint macrophages are critical for synovium and intra-articular fat pad homeostasis and OA development

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**INTRODUCTION:** Osteoarthritis (OA) is a degenerative joint disease characterized by chronic inflammation, cartilage degradation, and joint pain. Synovial macrophages are increasingly recognized as key contributors to OA pathogenesis due to their central role in mediating inflammation and tissue remodeling. Inside the joint, another tissue harboring macrophage is intra-articular fat pad (IAFP), a unique type of white adipose tissue (WAT). Because of their close proximity, similar pathological changes in OA, and sharing the same mesenchymal progenitors, synovium and IAFP have emerged as one integrated functional tissue. Prior studies of macrophages in OA development relied on systemic macrophage depletion or intra-articular injection of macrophage depletion drug, which cause significant off-target effects and lead to conflicting results. To elucidate the function of joint macrophages without side effects, we generated a mouse model that depletes colony-stimulating factor 1 (*Csf1*) specifically within the joint using a *Cre* driven by *Gdf5*, whose expression is limited to interzone during early joint development. Csf1 is a crucial growth factor for macrophage differentiation and survival. In this study, we examined the joint phenotypes of *Csf1 CKO* mice and investigated the role of joint macrophages in synovium/IAFP homeostasis and OA development.

**METHODS:** *Animals*- All animal work was approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. *Gdf5-Cre* mice were crossed with *Csf1flox/flox* mice or *Rosa-tdTomato (Td)* mice, to produce *Gdf5-Cre:Csf1flox/flox* (*Csf1 CKO*) and *Gdf5-Cre:Td* mice, respectively. *Csf1 CKO* and their *WT* siblings at 3 months of age received destabilization of the medial meniscus (DMM) surgery at right knee joints and were sacrificed 1 week or 2 months later for analysis. Left knee served as control. Only male mice were used for this study. *Histology*- Mouse knee joints were fixed with 4% paraformaldehyde (PFA) overnight, followed by 8% formic acid decalcification for 1-3 days. Some were processed for paraffin sections for Safranin-O/Fast green (SO/FG) or Hematoxylin and Eosin (H&E). The others were processed for whole-mount cryosections and stained with antibodies against F4/80, PERILIPIN, PDGFRα, and CGRP or dye BODIPY. Sagittal sections from the center of the joints were used for quantification using ImageJ. *CFU-F assay*- Synovium/IAFP were digested with 0.25% trypsin–EDTA for 30 min and 300 U/ml collagenase I for 45 min. Dissociated cells were seeded at 5000 cells/well in a 6-well plate. *Statistics*- Data are expressed as means±SD and analyzed by one-way ANOVA and unpaired, two-tailed Student's t-test.

**RESULTS**: Using Td as a reporter, we confirmed that *Gdf5-Cre* only labels joint tissues but not bone (Fig. 1). Analyzing a single-cell RNA-sequencing dataset of synovium/IAFP tissue (GSE231755) revealed that fibroblasts are the major producer of Csf1 (Fig. 2). In line with these, *Csf1 CKO* mice at 3 months of age displayed a drastically reduced number of macrophages in both synovium and IAFP, but not in bone marrow, as shown by F4/80 staining (Fig. 3). H&E staining and immunostaining of PDGFRα, a marker of mesenchymal cells, revealed that *CKO* mice have more fibroblasts in synovium/IAFP (Fig. 3). CFU-F assay showed a 1.5-fold increase in colony formation in *CKO* mice (n=3/group, p<0.01), suggesting that joint macrophages regulate mesenchymal progenitors in synovium/IAFP. After DMM, macrophages in synovium/IAFP were greatly increased in *WT* mice at 1 week and returned to baseline by 2 months (Fig. 4). However, this expansion was largely eliminated in *CKO* mice. At 1 week post DMM, adipocytes in IAFP, labeled by PERILIPIN, mostly disappeared (Fig. 4A) and were replaced by PDGFRα+ fibroblasts, especially αSMA+ myofibroblasts (Fig. 5). Abundant lipid droplets (BODIPY+), presumably released from adipocytes, were detected (Fig. 5). Interestingly, *CKO* mice had fewer myofibroblasts but more fibroblasts than *WT* mice, suggesting a role of macrophages in promoting myofibroblast differentiation. They also had fewer lipid droplets, implying that macrophages form a barrier to retain lipid within the tissue. To understand inflammation-related pain, we performed CGRP staining and found significantly reduced sensory neuron fibers in *CKO* mice at baseline and after OA surgery (Fig. 6). At 2 months post-DMM, *CKO* mice displayed attenuated cartilage degeneration and synovitis suggested by a 46.0% decrease in OARSI score and a 59.3% decrease in Synovitis score, respectively, compared to *WT*.

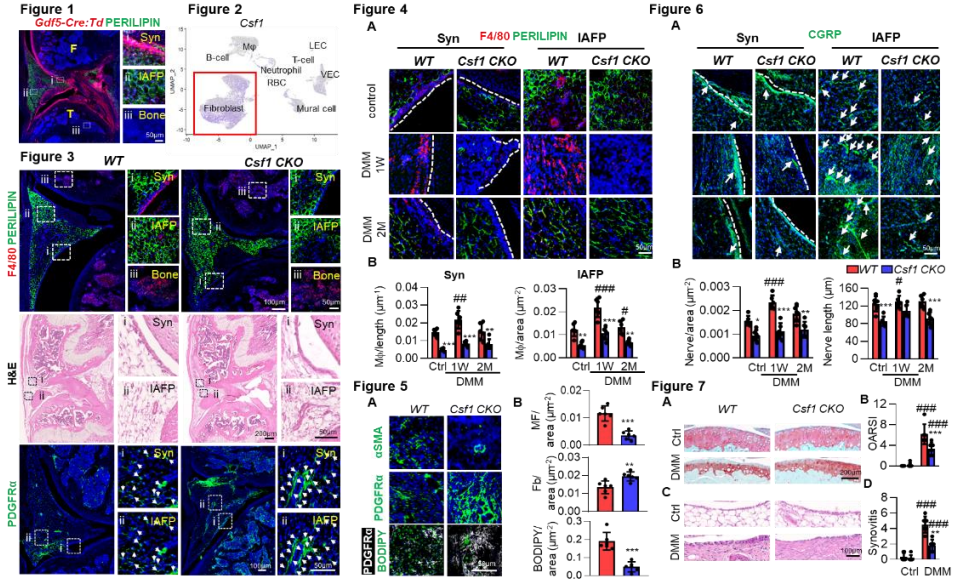


**Figure 1.** Fluorescence image of a knee joint from *Gdf5-Cre:Td* mice with PERILIPIN staining. Squares in the left low magnification image are shown at a high magnification at the right. Syn: synovium; F: femur; T: tibia. **Figure 2.** UMAP plot of *Csf1* expression in synovium/IAFP scRNA-seq dataset. VEC, vascular endothelial cells; LEC, lymphatic endothelial cells; Mφ, macrophages. **Figure 3.** Joint macrophage depletion increases fibroblasts in synovium/IAFP. Fluorescence (top: F4/80 and PERILIPI; bottom: PDGFRα) and H&E (middle) images of *WT* and *CKO* joints are shown. Arrows point to PDGFRα+ cells. **Figure 4.** Analysis of macrophages in control and OA joints. **(A)** F4/80 and PERILIPIN staining. **(B)** Quantification of macrophages in synovium/IAFP. n=6/group. **Figure 5.** Macrophages regulate myofibroblast formation at 1 week after DMM. **(A)** αSMA, PDGFRα, and BODIPY staining. **(B)** Myofibroblasts (αSMA+, MF), fibroblasts (PDGFRα+, Fb) and lipid droplets (BODIPY+) are counted. n=6/group. **Figure 6.** Analysis of neuron fibers in control and OA joints. **(A)** CGRP staining. **(B)** Quantification of nerve number and length in synovium/IAFP. n=6/group. **Figure 7.** Joint macrophage depletion attenuates OA progression at 2 months post surgery. **(A)** SO/FG staining of articular cartilage. **(B)** Quantification of OARSI scores. n=6/group. **(C)** H&E staining of synovium. **(D)** Quantification of synovitis scores. n= 6/group. *CKO* v.s. *WT*: *P < 0.05, **P < 0.01, ***P < 0.001. OA v.s. control: #P < 0.05, ##P < 0.01, ###P < 0.001.

**DISCUSSION**: This work has successfully established a joint-specific macrophage depletion mouse model. Macrophages are known for releasing inflammatory factors in OA. Here, we discovered that macrophages are critical for stimulating myofibroblast formation and retaining lipid droplets at early-stage post OA injury, two features likely linked to OA development later. Since macrophages are only depleted in joint but not in other tissues, our data undoubtfully demonstrate a key role of macrophages in promoting cartilage degradation and synovial inflammation. Future studies will focus on the crosstalk between joint macrophages and mesenchymal cells in synovium/IAFP under healthy and diseased conditions.

**SIGNIFICANCE:** Using a genetic mouse model, we demonstrate a critical role of macrophages in maintaining joint tissue homeostasis and driving OA progression.