Macrophage YAP/TAZ Signaling Mediates Mechanoregulation of Bone Fracture Repair

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Introduction: Bone tissue can heal without scarring, but current treatments for fractures remain limited in efficacy and applicability. Harnessing biological mechanisms of repair, particularly the interplay between inflammatory signals and mechanical cues, offers a promising strategy to accelerate healing. In the early phase of fracture repair, macrophages (MΦs) infiltrate the fracture site. This phase of fracture repair is also mechanically dynamic, and these mechanical conditions impact the course of repair. Specifically, while rigid fixation induces intramembranous repair, early mechanical loading induces repair by endochondral ossification. Mechanical cues are transduced by a number of signaling pathways, including the transcriptional regulators Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ). Our group has shown that YAP and TAZ mediate mechanotransduction in osteoblast-lineage cells during both bone development [1] and repair [2]. Recent studies also suggest that MΦs are actively sensitive to mechanical cues and that MΦ mechanotransduction can modulate tissue regeneration [3]. However, the role of macrophage mechanosignaling in bone fracture repair remains unclear. Here, we query whether ablation of YAP/TAZ signaling in macrophages modulates fracture repair in response to mechanical loading.

Methods: Bone fracture: Procedures were performed on skeletally mature male and female mice; all methods followed IACUC regulations. Femoral fractures were stabilized with external fixators (stiff: 18.1 N/mm; compliant: 3.2 N/mm). Early fracture cells (3 dpf) were isolated for scRNA-seq (GSE230260) (N=3–4/group). Triple Collagen reporter mice: (Col1-YFP; Col2-CFP; Col10-mCherry) marked osteoblasts, chondrocytes, and hypertrophic chondrocytes. F4/80 immunofluorescence localized macrophages in the callus. YAP/TAZ knockout mice: Macrophage-specific knockouts were generated using LysM-Cre. Fracture healing: At 2 weeks, femurs were analyzed by micro-CT and Saf O/Fast Green histology (N=6–12/group, both sexes). BMDM isolation: BMDMs were isolated from femora/tibiae of WT and YAP^{flox}/TAZ^{flox} mice (N=4-7/group, both sexes). In vitro recombination: TAT-Cre was applied to macrophage cultures for 6 h. Cell shape: Actin cytoskeleton was visualized using phalloidin staining, and cell morphology was quantified with CellProfiler. Phagocytosis: GFP–E. coli were added to cultures for 2 h, and uptake quantified by microplate reader. Statistics: Outcomes were compared using t-tests or 2-way ANOVA, with $p<0.05$ considered significant.

Results: MΦs in fracture repair: scRNA-seq showed that compliant fixation increased MΦ fractions at 3 dpf compared to stiff fixation or contralateral marrow **(Fig. 1A, B)**. MΦs rapidly invaded the fracture site, localizing to the hematoma at 4 dpf. By 7 dpf, they formed canopy-like structures over osteoblasts (Col1-YFP) and chondrocytes (Col2-CFP) in the soft callus. At 10–14 dpf, MΦs infiltrated the hard callus, associating with hypertrophic chondrocytes (Col10-RFP) during endochondral ossification **(Fig. 1C)**. MΦ-conditional YAP/TAZ deletion: Conditional deletion in MΦ did not affect cortical bone morphometry in intact adult bone (data not shown). The experimental design is shown in the graphical abstract **(Fig. 2A)**. At 2 weeks post-fracture, Saf O/Fast Green staining **(Fig. 2B)** revealed persistent cartilage in KO calluses, indicating impaired resolution between fixation groups. Consistently, microCT analysis **(Fig. 2C)** showed that WT mice exhibited the expected reduction in BV/TV with compliant fixation, whereas this effect was lost in KO mice. Effects of YAP/TAZ deletion on MΦ function: In vitro, YAP/TAZ deletion suppressed TEAD targets (*Cyr61, Ctgf*; **Fig. 3A**), increased *IL-6*, and reduced *Arg1* **(Fig. 3B)**. Phalloidin staining showed morphological changes: WT MΦs displayed protrusions and small bodies, while KO MΦs appeared larger and rounded **(Fig. 3C)**. Functionally, YAP/TAZ deletion impaired phagocytosis of GFP–E. coli **(Fig. 3D)**.

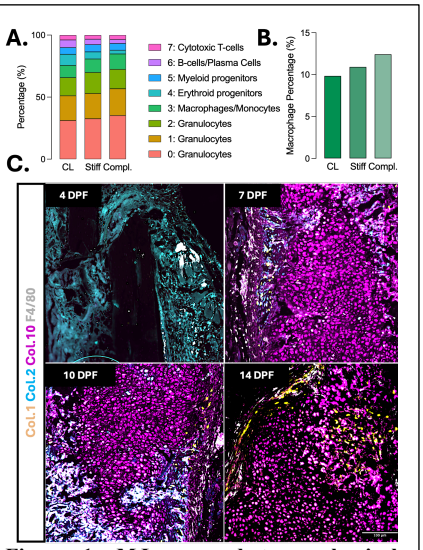

Figure 1: MΦ respond to mechanical stimuli in vivo. (A) scRNA-seq revealed eight subpopulations in the bone marrow. **(B)** Percentage of MΦs in contralateral unfractured (CL) limbs and in fractures with stiff or compliant fixation. **(C)** Immunofluorescence staining of the bone fracture callus. SB 100μm.

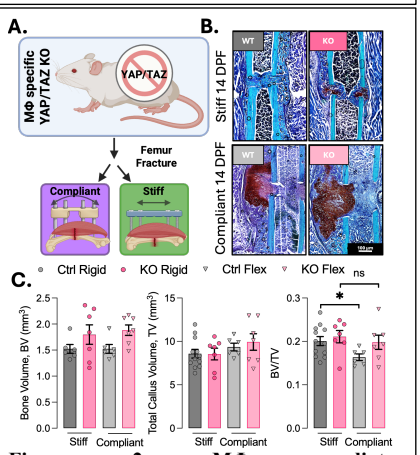

Figure 2. MΦs mediate mechanoregulation of fracture repair. (A) Experimental approach schematic. **(B)** Saf O staining of fractures with stiff vs. compliant fixation. SB 100μm. **(C)** Quantification of μCT parameters in fractured limbs. *p<0.05.

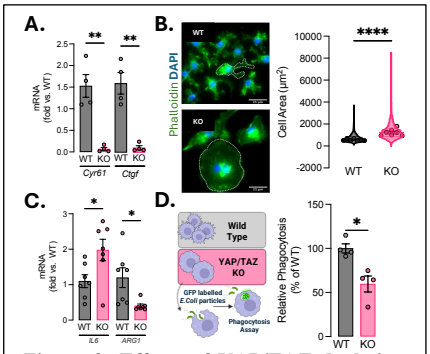

Figure 3: Effects of YAP/TAZ depletion on MΦ function. (A) qPCR of *Cyr61* and *Ctgf* expression in MΦs. (B) Actin cytoskeleton (phalloidin) and cell area quantification in of MΦs following TAT-Cre treatment. SB 25μm. **(C)** qPCR of *IL-6* and *Arg1* expression in MΦs. **(D)** Phagocytosis of GFP-labeled E. coli (% internalized GFP). *p<0.05, **p<0.01, ****p<0.0001.

Discussion: Our results reveal a critical role for YAP/TAZ signaling in macrophages in the mechanoregulation of fracture repair, shaping the cartilaginous callus milieu. MΦ-conditional YAP/TAZ deletion did not alter cortical bone morphometry, indicating that these signals are dispensable for skeletal development and homeostasis. During regeneration, however, MΦs infiltrated the callus early, co-localized with collagen-producing cells, and migrated dynamically between peripheral and central regions. Compliant fixation increased MΦ recruitment at 3 dpf, supporting mechanoregulated activation. Although cartilage callus formation was not abolished, YAP/TAZ deletion disrupted the mechanically driven endochondral response, consistent with WT MΦ localization to ossification sites. Mechanistically, we established an in vitro system using TAT-Cre to generate KO MΦs, showing that YAP/TAZ regulate cytoskeletal organization, cytokine profile, and phagocytic capacity. Together, these findings indicate that while YAP/TAZ signaling in MΦs is not required for skeletal development, YAP and TAZ direct MΦ recruitment during mechanoregulated fracture repair and regulate endochondral ossification. Ongoing studies will examine how MΦ mechanosignaling coordinates osteogenic–angiogenic coupling and functional repair.

Significance: Here we identify YAP and TAZ as mediators of MΦ activation by mechanical loading during bone repair and as regulators of MΦ state and function. Advancing our understanding of how mechanical and molecular cues regulate MΦ behavior may offer novel avenues for immunomodulatory and regenerative therapies.

References: [1] Collins+ *Dev. Cell. 2024*, **[2]** Kegelman+ *JBMR. 2020*, **[3]** Meli+ *Science Advances. 2020*.

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