Intra-Articular TAT-Cre-Mediated Deletion of Lats1/2 Induces Joint Pain and Synovial Dysfunction

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Introduction: The synovium is a bilayer membrane, composed primarily of fibroblasts and macrophages, that lines the joint capsule and produces synovial fluid.[1] During age-related and injury-induced osteoarthritis (OA) the synovium becomes fibrotic and stiffens. In response to this altered microenvironment, synovial cells become mechanoactivated:[2] fibroblasts transition to a myofibroblast-like phenotype, expressing fibrotic markers, and decreasing production of joint-lubricating factors,[3] while macrophages adopt a pro-inflammatory phenotype that exacerbates synovial dysfunction.[4] Although synovitis is known to correlate with pain and disease progression in OA,[5] the contribution of synovial pathology, and specifically that of mechanoactivated synovial cells, in the absence of cartilage damage or a destabilizing injury, remains unclear. To address this question, we focused on the Hippo pathway kinases, LATS1 and LATS2, which reduce nuclear translocation of the mechanoresponsive transcriptional co-activators YAP and TAZ. When present in the nucleus, YAP/TAZ can initiate fibrotic and inflammatory programs in synovial fibroblasts and macrophages, respectively.[3] Here, we delete LATS1/2 in the joint space via intra-articular injection of a membrane-permeable Cre recombinase to determine how YAP/TAZ gain-of-function affects synovial fibrosis, inflammation, and other joint pathologies, independent of an instigating injury.

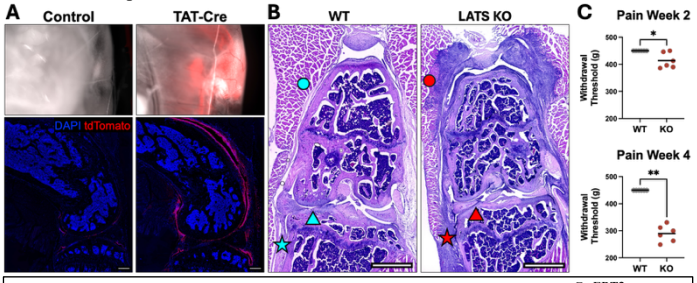

Figure 1. (A) Whole mount fluorescence and sections of Prg4^{CreERT2};R26R-tdTomato reporter knees 1 week after PBS or TAT-Cre injection. SB: 500μm. (B) WT and LATS1/2 KO knees at 4 weeks (H&E). Shapes indicate locations in Figs. 2-3. SB: 5mm. (C) Knee hyperalgesia at 2 and 4 weeks. *p<0.05, **p<0.01.

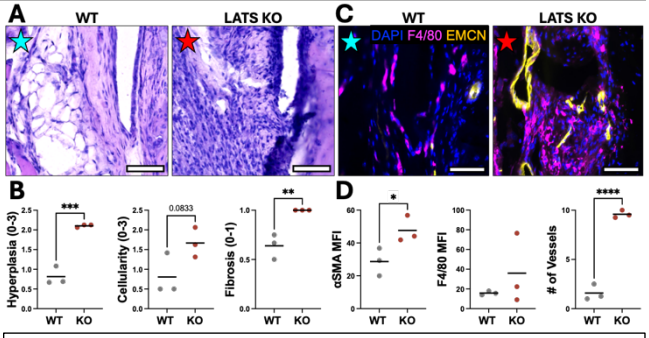

Figure 2. (A) H&E staining of synovium at 4 weeks. SB: 50μm. (B) Synovial scoring. (C) IF of synovium. SB: 50μm. (D) Quantification of αSMA and F4/80 staining intensity and vessel number. Stars indicate joint location from Figure 1. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Methods: All animal work was IACUC approved. Assessment of intra-articular recombination efficiency: Prg4^{CreERT2};R26R-tdTomato reporter mice received 1 intra-articular injection of PBS (n=3) or the recombinant, membrane-permeable (TAT-modified) Cre recombinase (TAT-Cre) (n=3). After 7 days, hind limbs were sectioned and analyzed for tdTomato signal. Intra-articular Lats1/2 deletion: Lats1$^{f/f}$;Lats2$^{f/f}$ (n=3) and WT (n=3) mice received 2 intra-articular TAT-Cre injections 48 hours apart. Only male mice were used as males develop more severe OA in surgical models.[6] Pain assessment: Mice were tested for joint hyperalgesia using a pressure application measurement device before TAT-Cre injection and weekly for 4 weeks post-injection. Micro-CT: At 4 weeks, joints were harvested and bone morphology was evaluated by micro-CT. Histological scoring: Synovial scoring[7] was performed on coronal H&E-stained cryosections. OARSI scoring[8] was performed on coronal Saf O/Fast Green-stained cryosections. Immunofluorescence: Joints were stained for alpha smooth muscle actin (αSMA), fibroblast activation protein (FAP), F4/80 (macrophage marker), and endomucin (EMCN, vessel marker). Fibrosis and macrophage infiltration were assessed by measuring average fluorescence intensity of αSMA, FAP, and F4/80. Vascularity was measured by counting EMCN-positive vessels per synovial gutter. Statistics: Outcomes were compared using t-tests.

Results: Intra-articular Lats1/2 ablation alters joint architecture and induces pain. Through intra-articular injections of TAT-Cre recombinase, we achieved precise local recombination in the synovium (**Fig 1A**). H&E staining of WT and Lats1$^{f/f}$;Lats2$^{f/f}$ knees 4 weeks after injection, revealed marked changes in joint architecture, particularly in the synovium and adjacent muscle (**Fig 1B**). By 2 weeks, Lats1$^{f/f}$;Lats2$^{f/f}$ mice had reduced withdrawal thresholds and this joint hyperalgesia increased at 3 and 4 weeks (**Fig 1C**). Lats1/2 ablation drives synovial hyperplasia and fibrosis. 4 weeks after TAT-Cre injection, Lats1$^{f/f}$;Lats2$^{f/f}$ mice displayed marked increases in lining hyperplasia, sublining cellularity, and synovial fibrosis (**Fig 2A,B**). IF revealed the accumulation of αSMA$^+$ fibroblasts and increased angiogenesis (EMCN$^+$ vascular structures) in the synovium (**Fig 2C,D**). Lats1/2 ablation increases subchondral bone resorption and promotes fibrotic remodeling of adjacent tissues. Micro-CT showed decreased bone volume and BV/TV in Lats1$^{f/f}$;Lats2$^{f/f}$ mice, while the cartilage was unaffected (**Fig 3A-D**). Histology of muscle showed centrally nucleated fibers and infiltration of macrophages and αSMA$^+$ fibroblasts (**Fig 3E-G**).

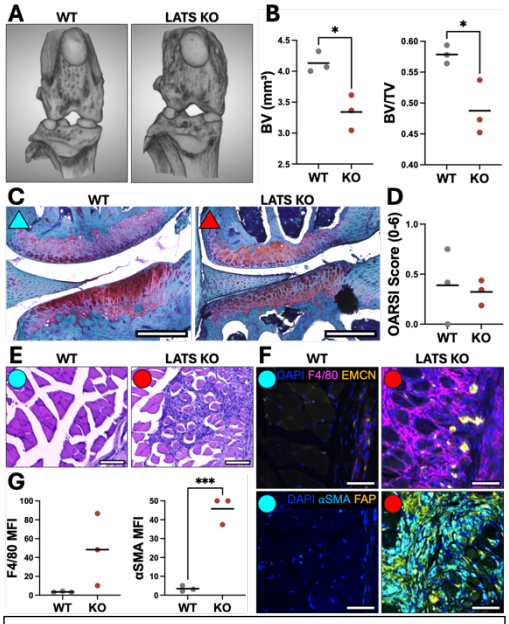

Figure 3. (A) Micro-CT. (B) Bone volume and density. (C) Cartilage staining (Saf O/Fast Green). SB: 250μm. (D) OARSI scoring. (E) H&E and (F) IF of muscle adjacent to the joint capsule. SB: 50μm. (G) Quantification of αSMA and F4/80 staining intensity. Triangles and circles indicate joint location from Figure 1. *p<0.05, ***p<0.001.

Discussion: We found that targeted intra-articular depletion of LATS1/2, suppressors of YAP/TAZ activation, induces synovitis and synovial fibrosis, as well as pathological alterations in the subchondral bone and adjacent muscle, in the absence of joint injury. We hypothesize Lats1/2 ablation mimics synovial mechanoactivation by increasing nuclear translocation of YAP/TAZ, as has been shown previously in nephron progenitor cells.[9] Our findings suggest mechanoactivation of synovial cells as a driver of joint pathology, even in the absence of joint damage. Intriguingly, the cartilage remained unaffected by Lats1/2 deletion, despite extensive changes in surrounding tissues; this may suggest that synovial dysfunction precedes cartilage erosion in the absence of an instigating injury. Future studies will include analyses of disease progression, tissue mechanics, and fibroblast–immune cell transcriptional signatures and crosstalk, defining how aberrant Hippo–YAP/TAZ signaling drives synovial fibrosis to impact joint health.

Significance/Clinical Relevance: Intraarticular Cre-mediated recombination revealed a profound impact of Lats1/2 knockout on joint pain and structure. This study establishes a role for Hippo–YAP/TAZ in synovial fibrosis and inflammation and supports the hypothesis that synovial pathology is an active contributor to disease progression in OA.

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References: 1. Scanzello, *Springer*, 2022; 2. Lemmon, *OAC*, 2024; 3. Bonnevie, *OAC*, 2024; 4. Knab, *Front Med*, 2022; 5. Mathiessen, *Arthritis Res Ther*, 2017; 6. Ma, *OAC*, 2007; 7. Obeidat, *OAC*, 2024; 8. Glasson, *OAC*, 2010; 9. McNeill, *JASN*, 2017.