Intra-Articular Non-Muscle Myosin II Knockout Mitigates Pain and Synovial Hyperplasia After Joint Injury

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Introduction: OA progression is characterized by changes in the mechanical properties of both the cartilage and synovium,[1,2] and these changes can impact mechanoactivation and alter the phenotype of resident cells.[3,4] Prior research has shown that disrupting mechanosignaling via intra-articular injection of either Rho or non-muscle myosin (NM-II) inhibitors improves cartilage integrity after destabilization of the medial meniscus (DMM).[4] While that study focused on the response of chondrocytes to such perturbations, the effects on other joint tissues and functional outcomes have not yet been explored. We recently developed a mouse model in which *Myh9* and *Myh10*, the genes encoding NM-IIA and NM-IIB, respectively, are both floxed allowing for their conditional deletion. Here, we sought to evaluate the effect of intra-articular NM-II ablation on cartilage, subchondral bone, and synovium, as well as pain behavior after joint injury.

Methods: All animal work was IACUC approved. DMM: To induce OA, DMM was performed on the right hind limb of Myh9$^{f/f}$;Myh10$^{f/f}$ (n=14) and WT mice (n=10). Only male mice were used as males develop more severe OA after DMM.[5] Intra-articular NM-II knockout: Mice received 2 bilateral intra-articular injections of PBS or TAT-Cre (a recombinant membrane-permeable Cre recombinase) 72 and 24 hours prior to DMM[6] (**Fig 1A**). Pain assessment: Joint hyperalgesia was assessed with a pressure application measurement device before DMM and 8 weeks after DMM. Micro-CT: 8 weeks post-DMM, 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. Fibroblast-like synoviocyte (FLS) isolation: FLS were isolated from the hind paws of WT mice. *In vitro* NM-II knockdown: FLS were transfected with non-targeting or *Myh9*- and *Myh10*-targeted siRNA, and knockdown was confirmed via RT-qPCR at 48 hours. Immunofluorescence: Cells were fixed and stained for actin, αSMA, FAP, and paxillin. Morphometric analysis: Cell morphometry was analyzed using CellProfiler. RT-qPCR: Expression of αSMA (*Acta2*) and fibronectin (*Fn*) was assessed by RT-qPCR. Statistics: Outcomes were compared by two-way ANOVA with Tukey's post-hoc comparisons.

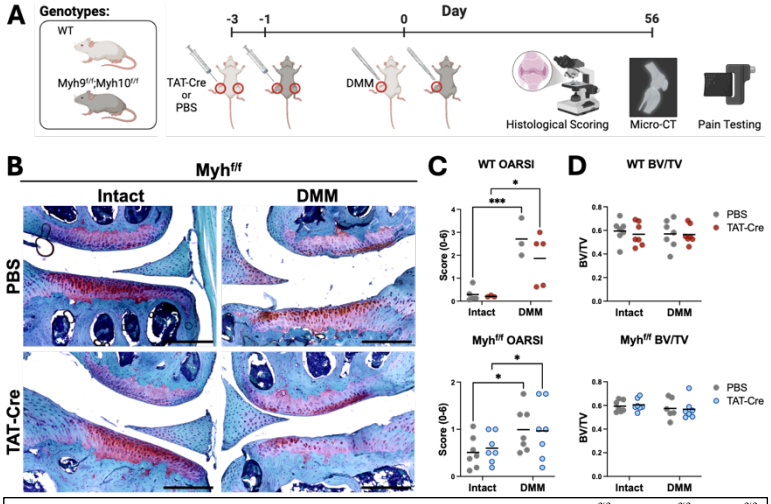

Figure 1. (A) Study design. (B) Saf O/Fast Green staining of Myh9$^{f/f}$;Myh10$^{f/f}$ (Myh$^{f/f}$) mice 8 weeks post-DMM. Scale: 250μm. (C) OARSI scoring of WT and Myh$^{f/f}$ joints. (D) Quantification of subchondral bone density via micro-CT. *p<0.05, ***p<0.001.

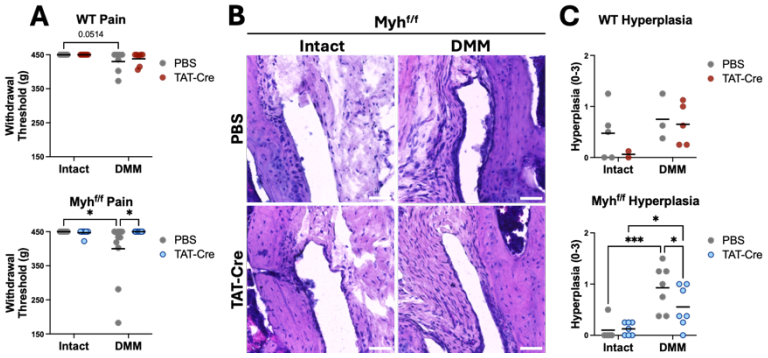

Figure 2. (A) WT and Myh9$^{f/f}$;Myh10$^{f/f}$ (Myh$^{f/f}$) knee hyperalgesia 8 weeks post-DMM. (B) Histological scoring of synovial hyperplasia. (C) H&E of Myh9$^{f/f}$;Myh10$^{f/f}$ synovium. Scale: 50μm. *p<0.05, ***p<0.001.

Results: Intra-articular NM-II ablation does not alter cartilage or subchondral bone morphology after DMM. TAT-Cre injection did not alter cartilage pathology, as OARSI scoring 8 weeks after DMM showed significant cartilage pathology in both WT and Myh9$^{f/f}$;Myh10$^{f/f}$ mice (**Fig 1B-C**). NM-II ablation also did not alter subchondral bone volume or density (**Fig 1D**). NM-II knockout mitigates pain and synovial pathology after joint injury. In WT mice, DMM reduced the pressure required to elicit paw withdrawal, indicating increased pain, with no difference observed when comparing PBS and TAT-Cre injected mice. Conversely, in Myh9$^{f/f}$;Myh10$^{f/f}$ mice, TAT-Cre injected mice had significantly higher withdrawal thresholds than PBS controls, indicating decreased pain with NM-II knockout (**Fig 2A**). TAT-Cre also reduced lining hyperplasia in Myh9$^{f/f}$;Myh10$^{f/f}$ synovium compared to PBS controls, while having no effect in WT mice (**Fig 2B-C**). NM-II knockdown disrupts FLS mechanoactivation. To investigate a potential mechanism underpinning these changes, we explored the effect of NM-II knockdown on FLS *in vitro*. Treatment with *Myh9*- and *Myh10*-targeted siRNA reduced cell spreading, increased aspect ratio, and decreased expression of αSMA and fibronectin, even under conditions that promote FLS mechanoactivation (TGFβ supplementation) (**Fig 3 A-C**).

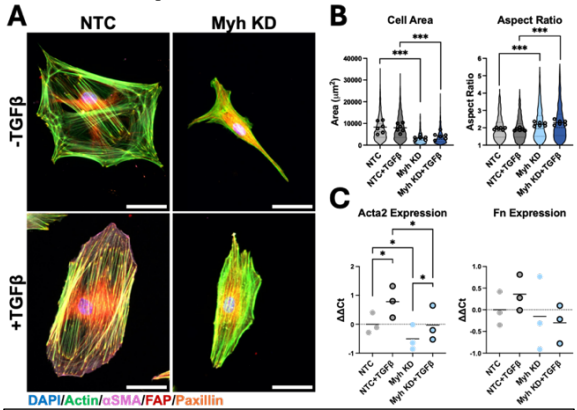

Figure 3. (A) IF of FLS without or with Myh knockdown. Scale: 50μm. (B) Quantification of cell morphology. (C) Gene expression. *p<0.05, ***p<0.001.

Discussion: Our findings demonstrate that intra-articular NM-II ablation mitigates joint hyperalgesia and lessens synovial pathology, despite persistent cartilage damage. This suggests that NM-II–mediated synovial mechanotransduction may facilitate pathological remodeling and pain in OA. Our *in vitro* data further support this, as NM-II knockdown disrupted morphological signs of mechanoactivation and expression of fibrosis-related genes. While the lack of cartilage protection was surprising given previous data on the efficacy of pharmacologic NM-II inhibition,[4] this may be due to potential poor penetration of TAT-Cre into cartilage. Ongoing work aims to understand the efficiency of TAT-Cre in various joint tissues. Future studies will also include additional functional outcomes, explore tissue micromechanics, and utilize scRNA-seq and co-culture systems to characterize cell and tissue crosstalk in the context of injury.

Significance/Clinical Relevance: This study identifies NM-II as a driver of synovial pathology and pain in OA and indicates that targeting synovial cell mechanoactivation may ameliorate OA symptoms, independent of effects on the cartilage and bone.

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References: 1. Stolz, *Nature Nanotech*, 2009; 2. Kim, *ORS*, 2024; 3. Bonnevie, *OAC*, 2024; 4. Kim, *PNAS*, 2015; 5. Ma, *OAC*, 2007; 5. Bernstein, *ORS*, 2025; 6. Obeidat, *OAC*, 2024; 7. Glasson, *OAC*, 2010.