

SM04690, a Wnt Pathway Inhibitor: Anti-Inflammatory and Cartilage Protective Effects in Preclinical OA Models

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Background

- Knee osteoarthritis (OA) is characterized by degradation of articular cartilage, subchondral bone remodeling, synovitis, and inflammation.^{1,2}
- Increased Wnt signaling has been linked to inflammation and OA pathogenesis.^{2,3}
- Samumed is developing a small molecule Wnt pathway inhibitor, SM04690, as a potential therapeutic administered as an intra-articular (IA) treatment for knee OA.
- In previous preclinical studies, SM04690 inhibited inflammation, decreased cartilage degradation, regenerated cartilage, and demonstrated sustained local exposure with no observed systemic toxicity.⁴
- The current studies characterized SM04690 anti-inflammatory effects *in vitro* and in an *in vivo* OA model.

Methods

- **Experiment 1: Primary anti-inflammatory activity:** Evaluated by measuring TNF- α and IL-6 secretion using ELISA in primary PBMCs. A panel of pro- and anti-inflammatory cytokines (TNF- α , IL-1 α , IL-1 β , IL-2, IL-6, IL-8, IL-17A, IL-17F, IFN- γ , PGE2) was evaluated by a) ELISA; b) T and B cell proliferation by flow cytometry in PBMCs, and c) T and B cell co-cultures stimulated with superantigen (sAg) or lipopolysaccharides (LPS), compared with vehicle (DiscoverX BioMAP® platforms).
- **Experiment 2: Mechanism of action:** SM04690 effects on LPS-induced expression and phosphorylation of NF κ B in THP-1 cells were evaluated by qPCR and Western Blot.
- **Experiment 3: OA model (*in vivo*):** SM04690 activity was evaluated in a rat monosodium iodacetate (MIA) injection-induced model of OA, followed by single IA SM04690 or vehicle injection at Day 3.
 - Joint inflammation was evaluated by H&E staining, synovial thickness measurement, and qPCR for pro-inflammatory markers (TNF- α , IL-1 β , IL-16).
 - Cartilage protection was measured by qPCR for MMPs and ADAMTS5 at Day 11. Cartilage appearance was evaluated by Safranin-O staining and Osteoarthritis Research Society International (OARSI) scoring at Day 28.⁵
 - Pain was measured as paw withdrawal threshold using Von Frey apparatus.

Results

Experiment 1: SM04690 suppressed inflammatory cytokines compared with vehicle

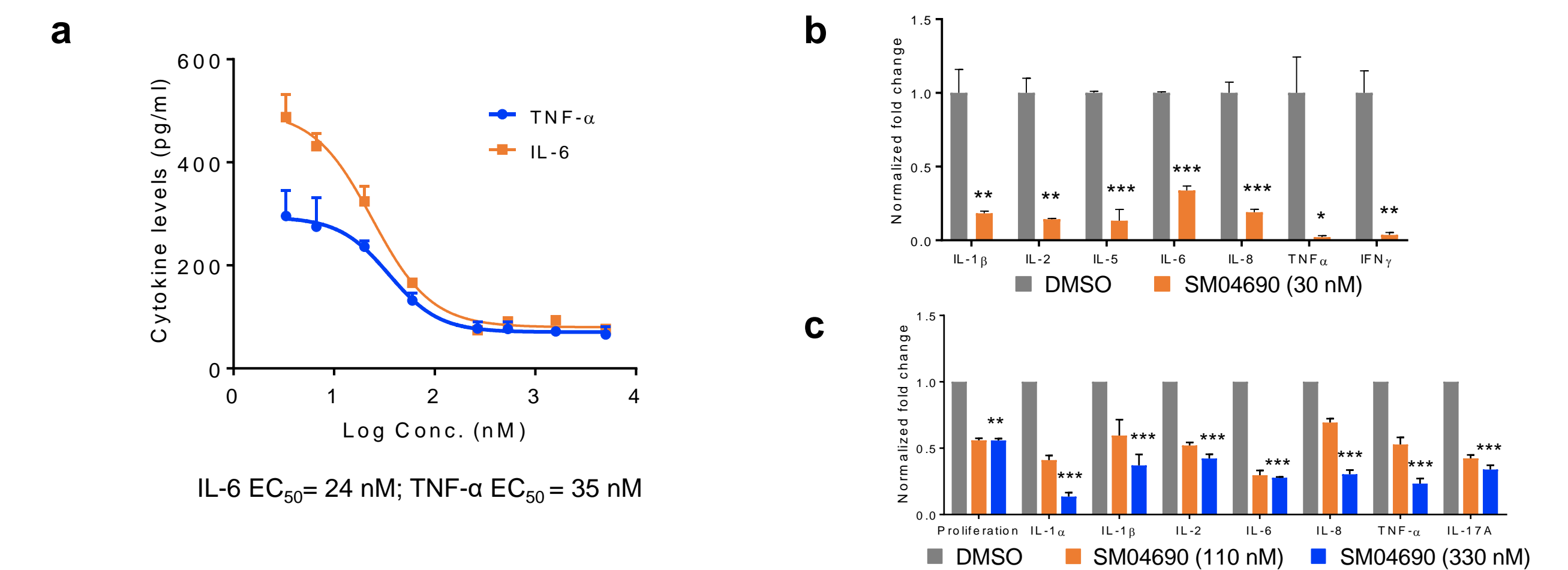


Figure 1. Synovial fibroblasts: dose-dependent inhibition of IL-6 (a) and TNF- α (a) production demonstrated in both cell types. SM04690 inhibited pro-inflammatory cytokine secretion compared with vehicle as shown in (b) and (c) (n=3 replicates, mean \pm SEM, *p<0.05, **p<0.01, ***p<0.001).

Experiment 2: SM04690 inhibited NF κ B phosphorylation and expression in human monocytes stimulated with LPS

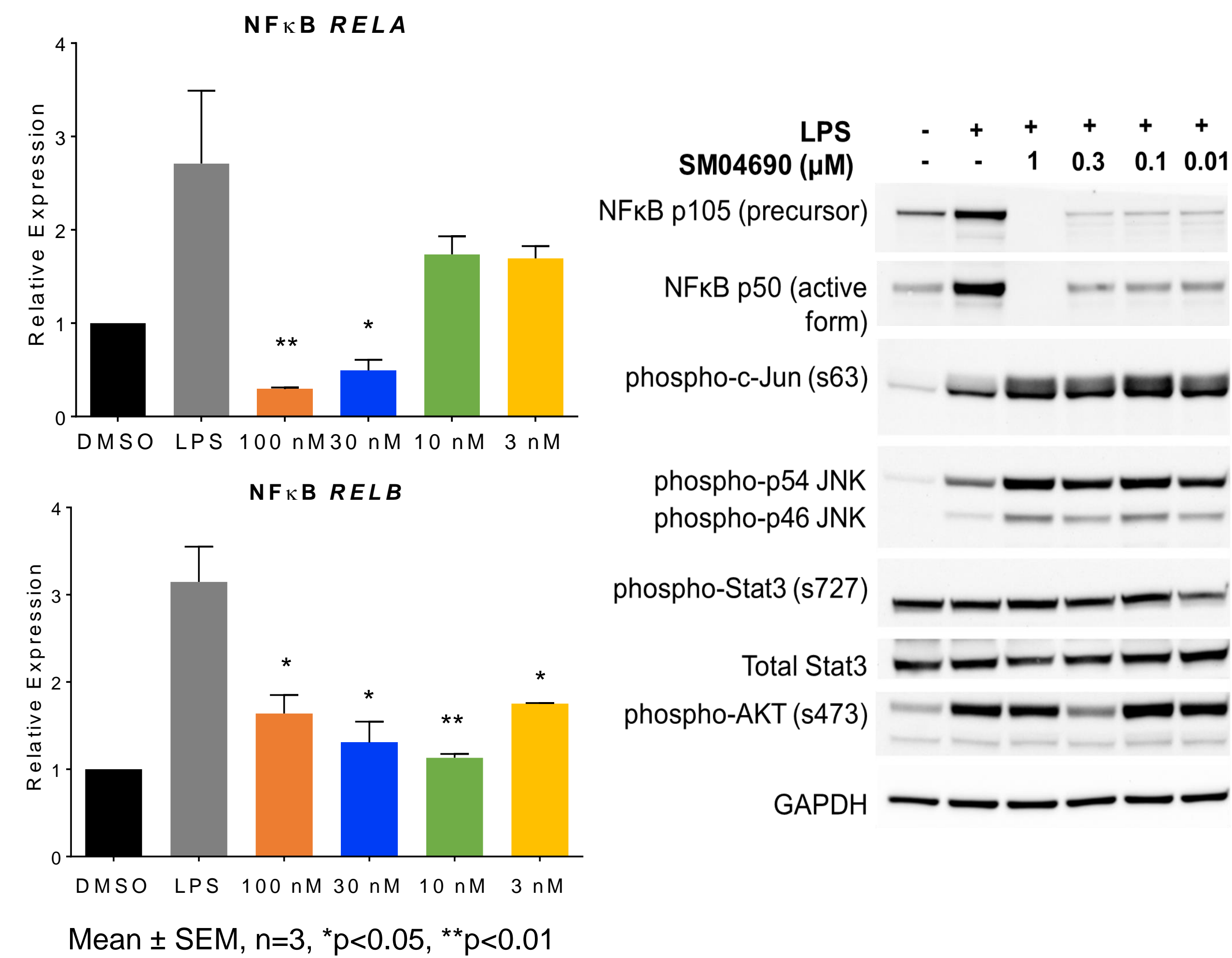


Figure 2. SM04690 specifically inhibited NF κ B phosphorylation and expression *in vitro*.

Experiment 3: SM04690 attenuated inflammation in a rat MIA knee OA model compared with vehicle

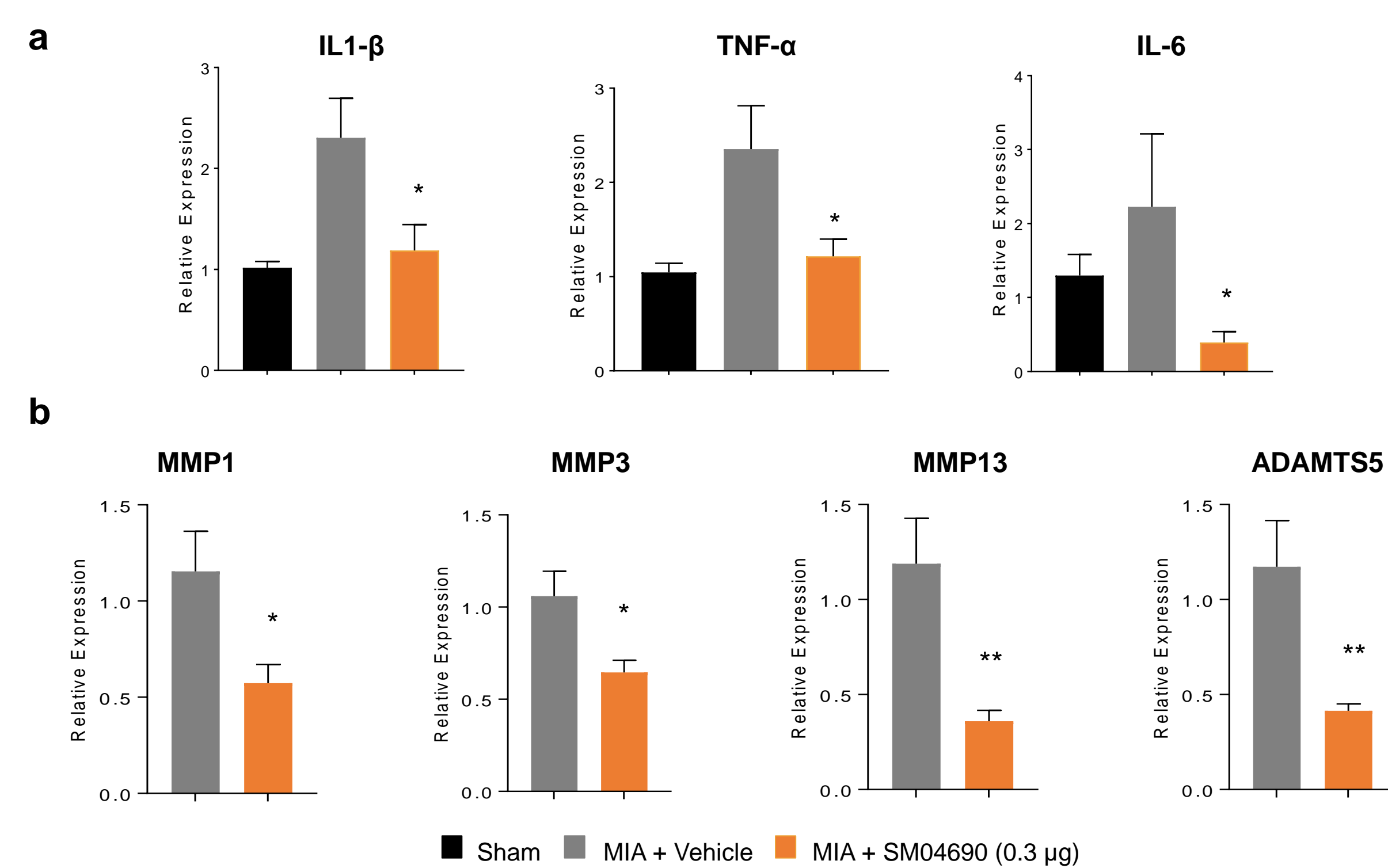


Figure 3. Single IA injection of SM04690 decreased inflammatory cytokines (n=10 rats/group, mean \pm SEM, *p<0.05, one-way ANOVA) (a) and matrix metalloproteinases (MMPs) (=8 rats/group, Mean \pm SEM, *p<0.05, **p<0.01, t-test) (b) compared with vehicle at Day 11.

Results

Experiment 3: SM04690 attenuated acute inflammation in a rat MIA knee OA model compared with vehicle

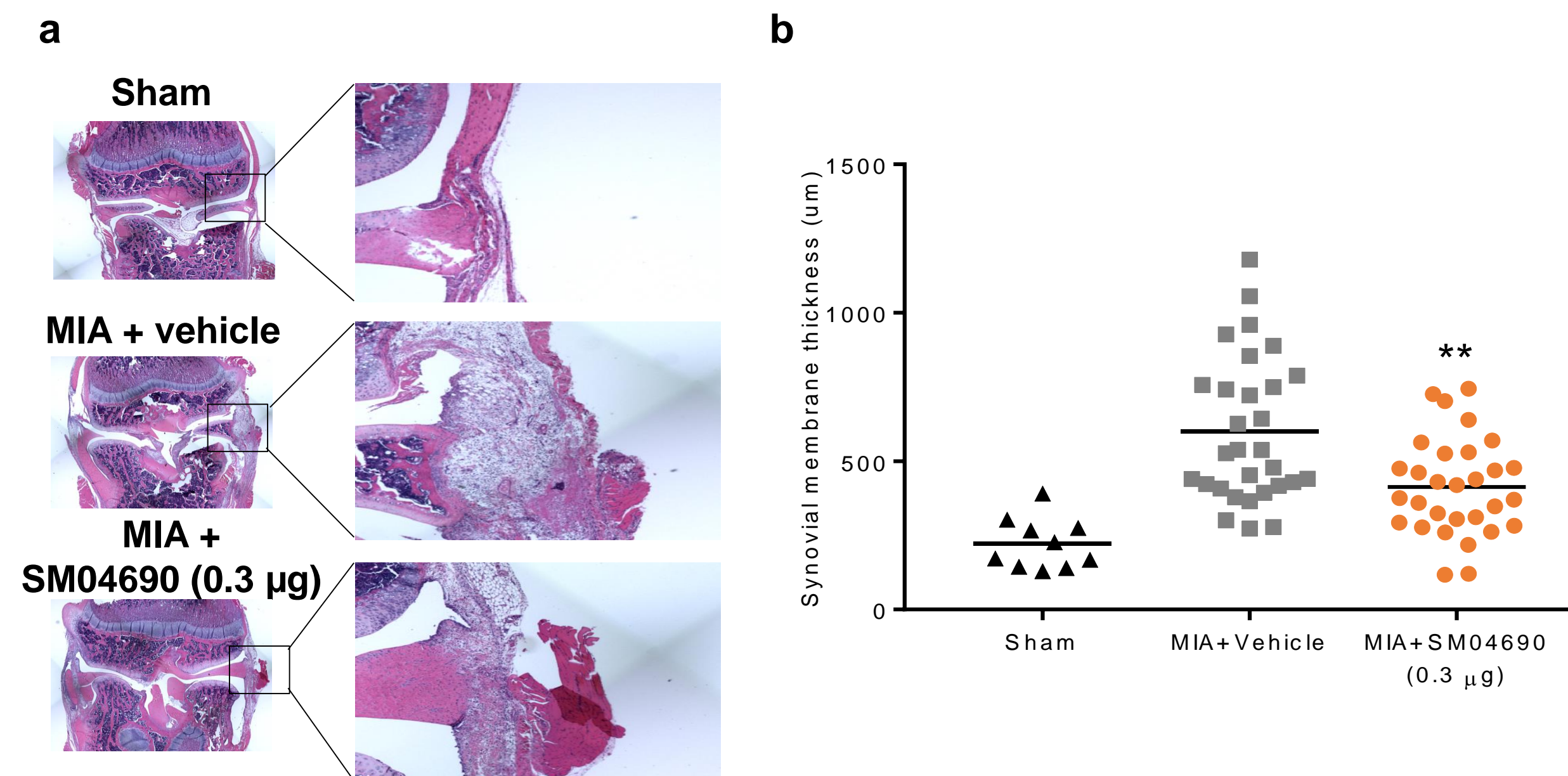


Figure 4. (a) H&E staining after a single IA injection of SM04690 demonstrated decreased inflammatory infiltrates, decreased hypercellularity, and improved structural integrity compared with vehicle treatment at Day 11. (b) Synovial membrane thickness was significantly decreased in SM04690 joints compared with vehicle at Day 11 (n=30 sections, mean \pm SEM, **p<0.01, one-way ANOVA).

Experiment 3: SM04690 protected cartilage in a rat MIA knee OA model compared with vehicle

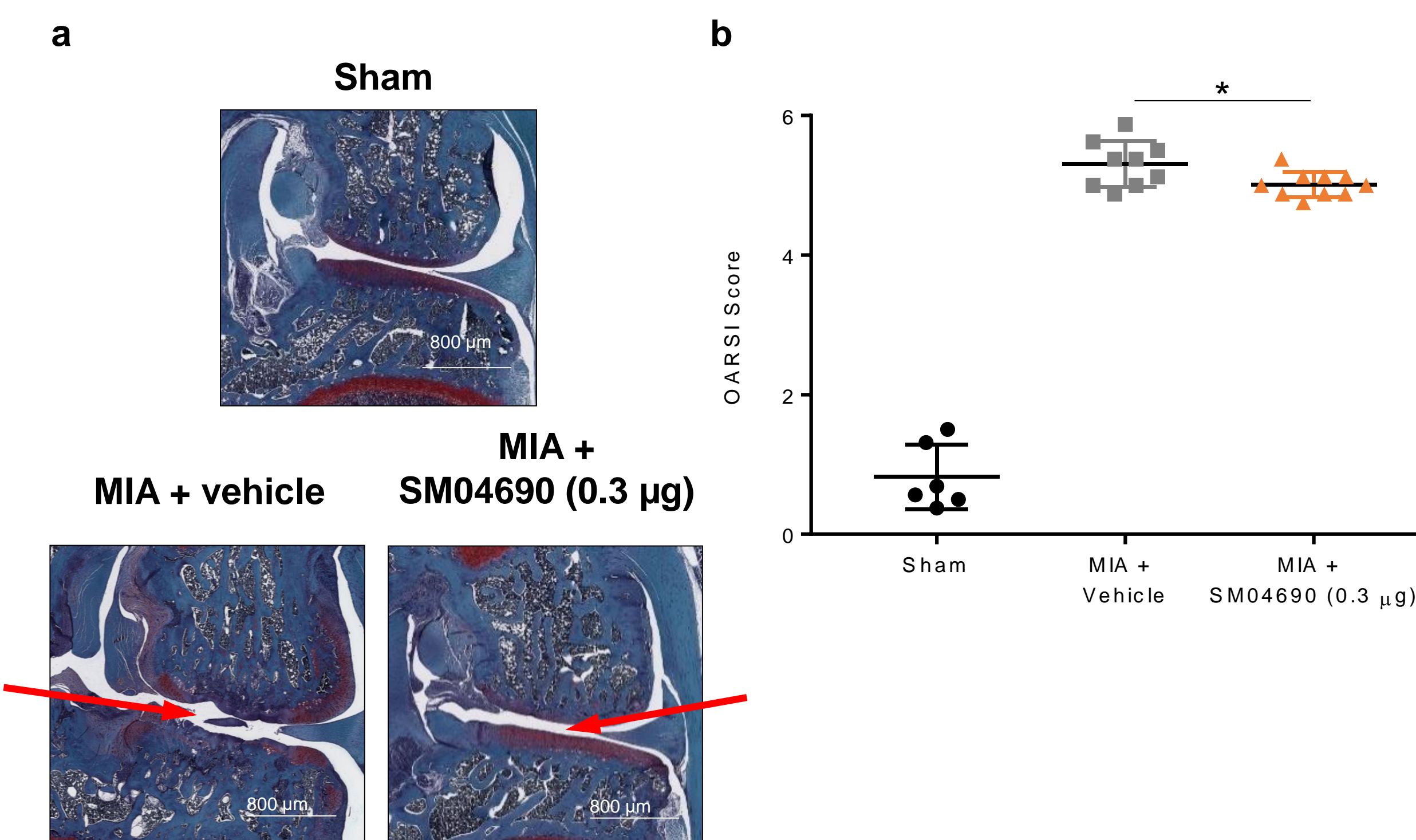


Figure 5. Single IA injection of SM04690 improved Safranin-O staining (a) and OARSI scores (b) at Day 28 comparing treatment with vehicle (n= 10, mean \pm SD, *p<0.05, Mann-Whitney U test).

Experiment 3: SM04690 attenuated pain in a rat MIA knee OA model compared to vehicle

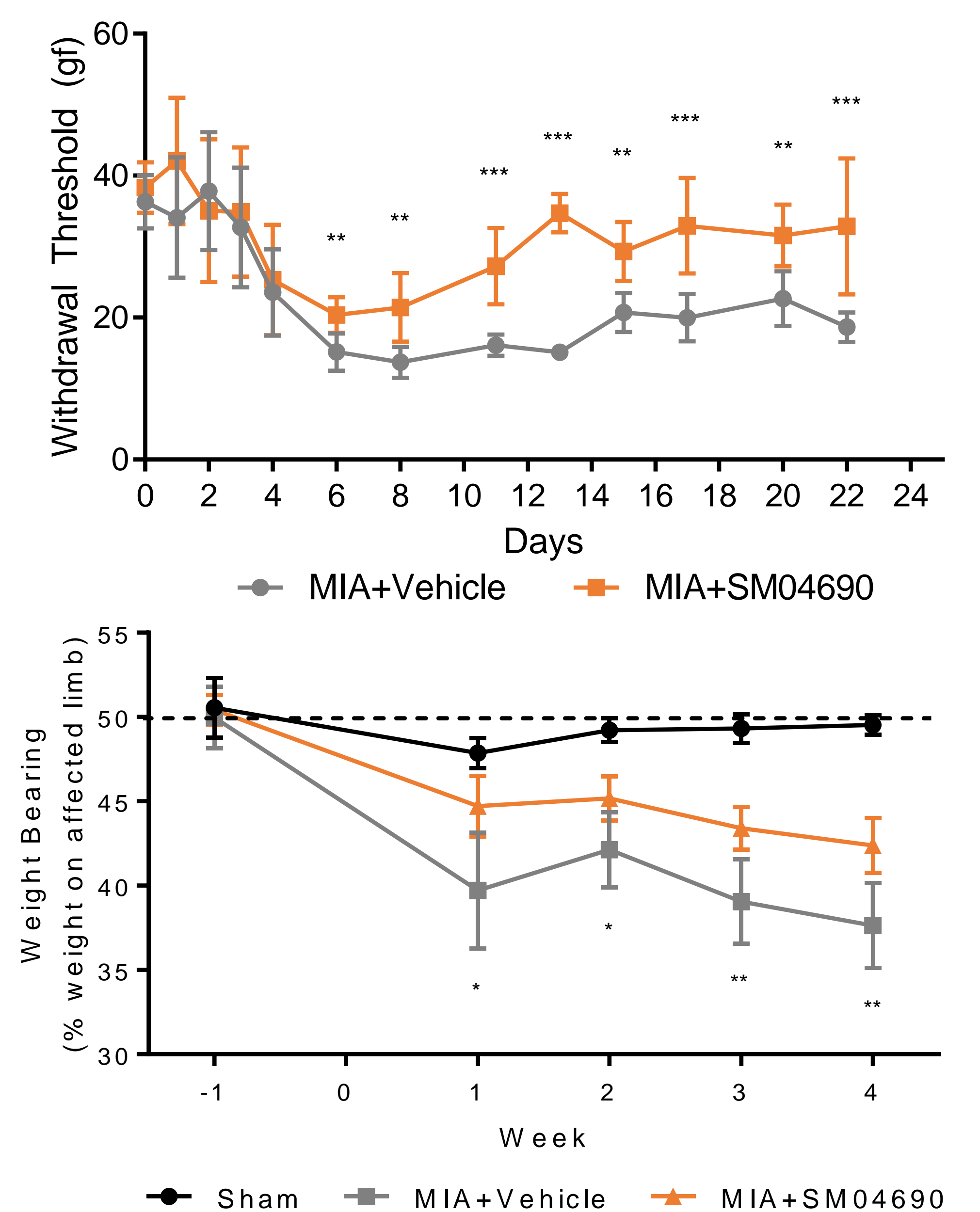


Figure 6. Single IA injection of SM04690 decreased pain (measured by Von Frey) (a) and improved gait (measured as weight distribution) (b) compared to vehicle treatment (n=10, estimated treatment effect \pm 95% CI, *p<0.05, **p<0.01, ***p<0.001, generalized estimating equation regression).

Conclusions

- *In vitro*, SM04690 demonstrated potent anti-inflammatory effects across a broad range of cytokines.
- In an MIA knee OA model, SM04690 attenuated inflammation, improved pain, and protected cartilage.
- Studies to further investigate the anti-inflammatory mechanism of action for SM04690 are ongoing.
- A human phase 2b clinical trial is in progress (clinicaltrials.gov identifier NCT03122860).

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