

Discovery of a Small Molecule Inhibitor of the Wnt Pathway (SM04690) as a Potential Disease Modifying Treatment for Knee Osteoarthritis

samumed

Poster# 0503

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Background

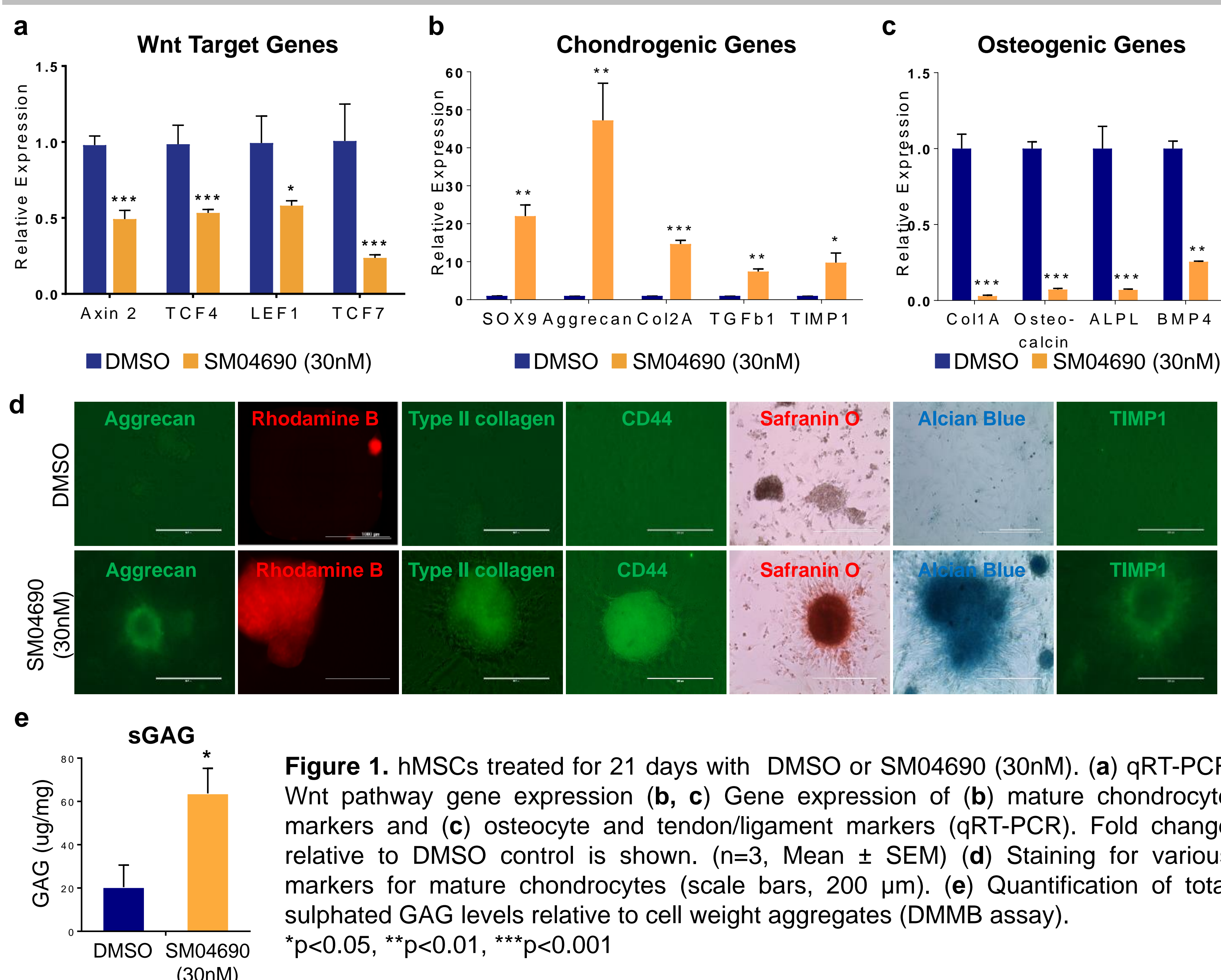
- Knee osteoarthritis (OA) is characterized by destruction of articular cartilage, subchondral bone alterations, and synovitis.¹
- At a cellular level, Wnt signaling affects OA pathogenesis in joints by influencing inflammation, cartilage breakdown, and bone / cartilage formation. Increased Wnt signaling induces stem cells to differentiate into osteoblasts, and decreased signaling induces chondrogenesis.²
- Samumed is developing a small molecule Wnt pathway inhibitor, SM04690, as a potential disease modifying OA drug (DMOAD) injected into the knee.
- Preclinical studies of SM04690 were conducted to evaluate chondrogenesis, anti-inflammation, cartilage protection, and joint health.

Methods

- Wnt pathway inhibition was measured by qPCR in human mesenchymal stem cells (hMSCs).
- Chondrogenesis was evaluated using hMSCs by qRT-PCR and immunocytochemistry.
- Cytokine induced protease release and glycosaminoglycan (GAG) breakdown in chondrocytes was measured by qRT-PCR and dimethylmethylene blue (DMMB) assay.
- Anti-inflammatory activity was evaluated by measuring TNF- α and IL-6 secretion using ELISA in synovial fibroblasts stimulated with IL-1 β . Pro-inflammatory cytokines (TNF- α , IL-1 α , IL-1 β , IL-2, IL-6, IL-8, IL-17A, IL-17F, IFN- γ , & PGE2) were evaluated by ELISA in T and B cell co-cultures stimulated with superantigen or LPS, compared to vehicle or two benchmark immunosuppressants (cyclosporin A and prednisolone).
- Pharmacokinetics of SM04690 in plasma and joint were evaluated following intra-articular (IA) injection in rats.
- In vivo* activity of SM04690 was evaluated in a rat model: anterior cruciate ligament transection with medial meniscal tear (ACLT+pMMx) using Osteoarthritis Research Society International (OARSI) scoring and biomarker measurement in knee and plasma by qPCR and ELISA.

Results

SM04690 inhibited Wnt signaling and induced chondrocyte differentiation in hMSCs *in vitro*



SM04690 protected chondrocyte from catabolic breakdown *in vitro*

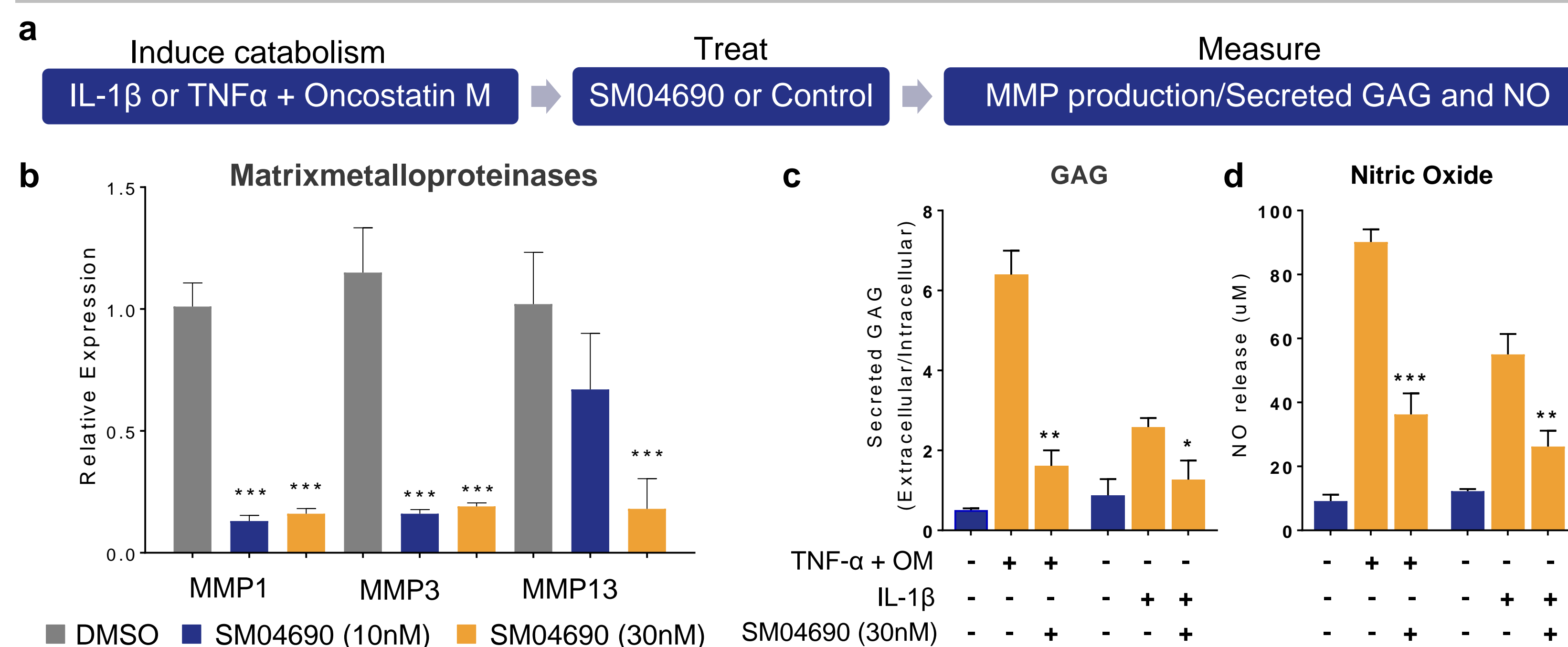
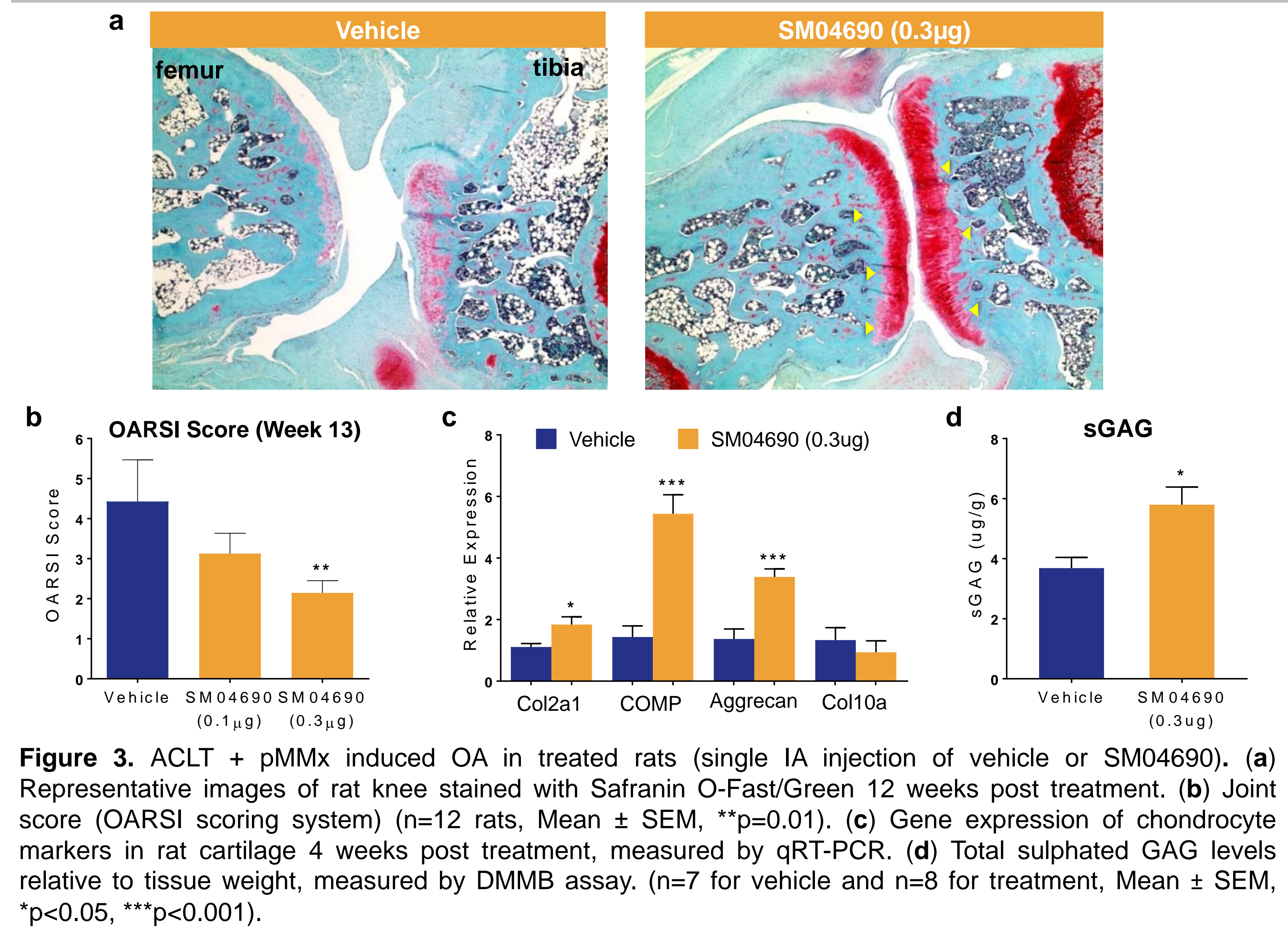


Figure 2. (a) Schematic of chondrocytes treated with cytokines TNF- α (20ng/ml) + Oncostatin M (10ng/ml) or IL-1 β (10ng/ml) and SM04690 (30nM) or vehicle for 72hrs. (b) Gene expression of proteases (MMP1, MMP3, MMP13, IHH), measured by qRT-PCR. (c) Levels of secreted GAG expressed as ratio of intracellular GAG measured by DMMB assay. (n=3, Mean \pm SEM). (d) Levels of secreted Nitric Oxide measured using Griess reagent assay. (n=6, Mean \pm SD, *p<0.05, **p<0.01, ***p<0.001).

Results

SM04690 regenerated cartilage in the ACLT+pMMx model of rat OA



SM04690 protected cartilage in the ACLT+pMMx model of rat OA

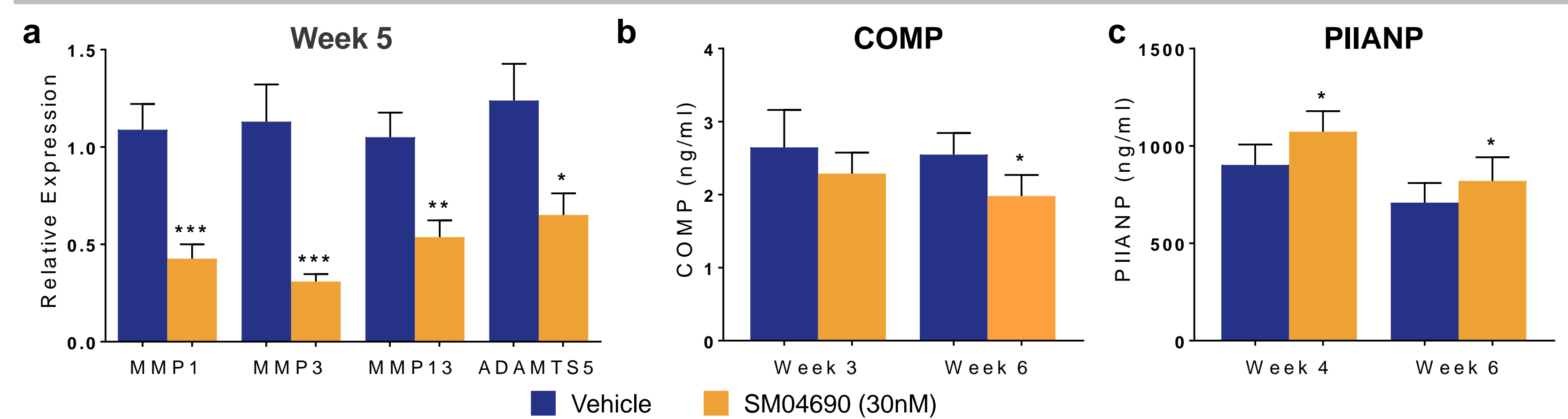


Figure 4. ACLT + pMMx induced OA in treated rats (single IA injection of vehicle or SM04690). (a) Protease gene expression in rat cartilage 4 weeks post treatment (qRT-PCR) (n=7 vehicle, n=8 treatment, Mean \pm SEM, *p<0.05, **p<0.01, ***p<0.001). (b, c) Circulating COMP and PIIANP measured by ELISA (n=12 rats, Mean \pm SEM, *p<0.05).

SM04690 inhibited inflammatory responses in co-culture systems *in vitro* with comparable or greater potency than Cyclosporin A and Prednisolone

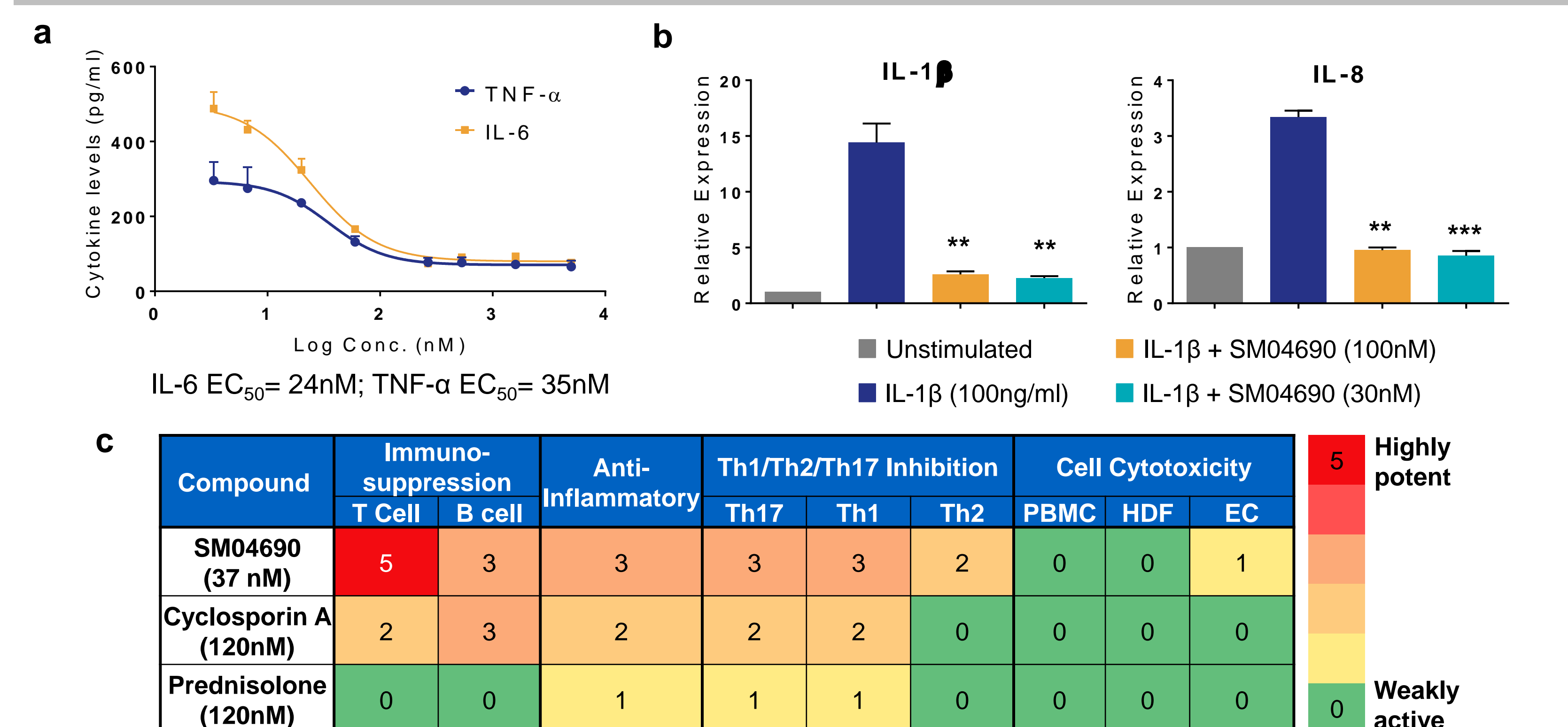


Figure 5. (a) IL-6 and TNF- α inhibition in human synovial fibroblasts stimulated with IL-1 β and treated with SM04690 for 24hrs measured by ELISA. (b) Inflammatory cytokine inhibition in human synovial fibroblasts stimulated with IL-1 β and treated with SM04690 for 24hrs (qRT-PCR) (n=3, Mean \pm SEM, *p<0.05, **p<0.01, ***p<0.001) (c) Comparison of *in vitro* anti-inflammatory activity of SM04690 with cyclosporin A and prednisolone performed on DiscoverX BioMAP[®] platform (scale 0-5; 0=weak and 5=highly potent activity). SM04690 demonstrated comparable or greater potency than the two standard-of-care drugs across several anti-inflammatory assays.

Conclusions

- SM04690, a small molecule Wnt pathway inhibitor, induced chondrogenesis, protected chondrocytes from catabolic breakdown, increased cartilage thickness and improved joint health in a rat model of knee OA.
- Additionally, potent anti-inflammatory effects of SM04690 observed in various cell types may provide beneficial effects in the treatment of OA.
- Human clinical trials with SM04690 are ongoing.

References

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