Bhattacharjee et al.:

A Human Osteoarthritis Mimicking Goat Cartilage Explant-Based Disease Model for Drug Screening

Supplementary Data

Tab. S1: Age, gender, and grade of OA of all the patients included in the study

Serial no.	Gender	Age	OA grade
1	M	62	4
2	F	55	3
3	M	65	4

Tab. S2: Details of the antibody used in the study

Antibody	Source	Catalog no.	Concentration /dilution	Clone/isotype
Anti-Collagen II	DSHB	II-II6B3	6.00 µg/mL	Monoclonal/IgG1
Anti-Chondroitin sulfate	DSHB	9BA12	6.20 µg/mL	Monoclonal/IgM
Anti-Aggrecan	DSHB	12/21/1-C-6	6.40 µg/mL	Monoclonal/IgG1
Anti-Collagen X	Sigma Aldrich	C7974	4.25 µg/mL	Monoclonal/IgM
Anti-MMP13	Sino Biologicals	102015-T10	1:250	Polyclonal/IgG
Anti-ADAMTS4	Sino Biologicals	106767-T08	1:250	Polyclonal/IgG
Anti-iNOS	Sino Biologicals	102279-T02	1:250	Polyclonal/IgG
Anti-p38	Sino Biologicals	10081-MM07	1:100	Monoclonal/IgG2/Clone#7
Anti-rabbit IgG	Jackson Immuno Research	111-165-003	2.00 µg/mL	Polyclonal/IgG
Anti-mouse IgG/M	Jackson Immuno Research	115-545-044	2.00 µg/mL	Polyclonal/IgG+IgM

Tab. S3: List of primer sequences for qPCR

Name	Sequence
MMP13	F: 5'-GAGCACTCATGTTTCCCATCTA-3' R: 5'-GAGACTGAATCCCTTGGACATC-3'
ADAMTS4	F: 5' CTGACCTCTTCAAGAACTTCCC 3' R: 5' GTGGGTCCAGCACGTAATAG 3'
COL10A1	F: 5' TGCTTTCACTGTTATCCTCTCC 3' R: 5' TCCAGTTCTTGGGTCGTAGTAATG 3'
RUNX2	F: 5' CTTCACAAATCCTCCCCAAGTAGCTACC 3' R: 5' GGTTTAGAGTCATCAAGCTTCTGTCTGTG 3'

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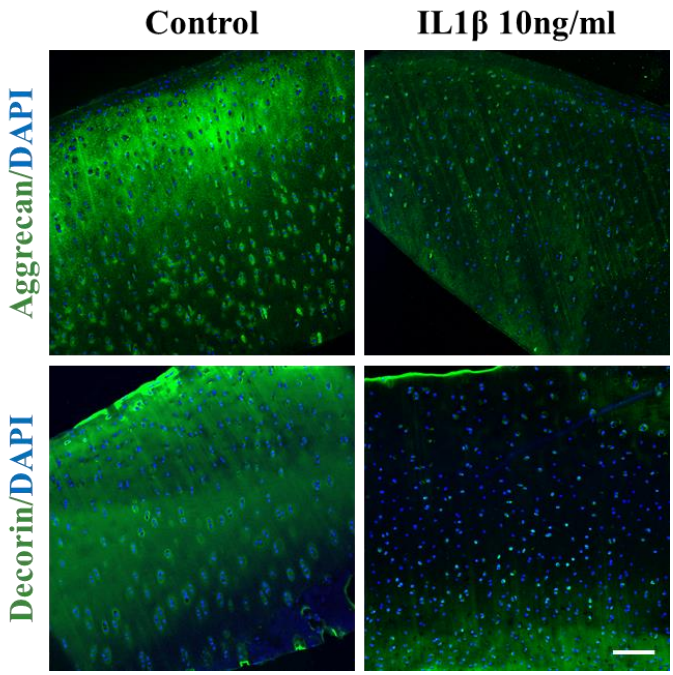

Fig. S1: Effect of IL1β treatment on cartilage matrix markers

Immunofluorescence micrographs of goat cartilage explant sections stained for aggrecan (green, top), decorin (green, bottom), and nuclei (blue) (scale bar – 100 µm).

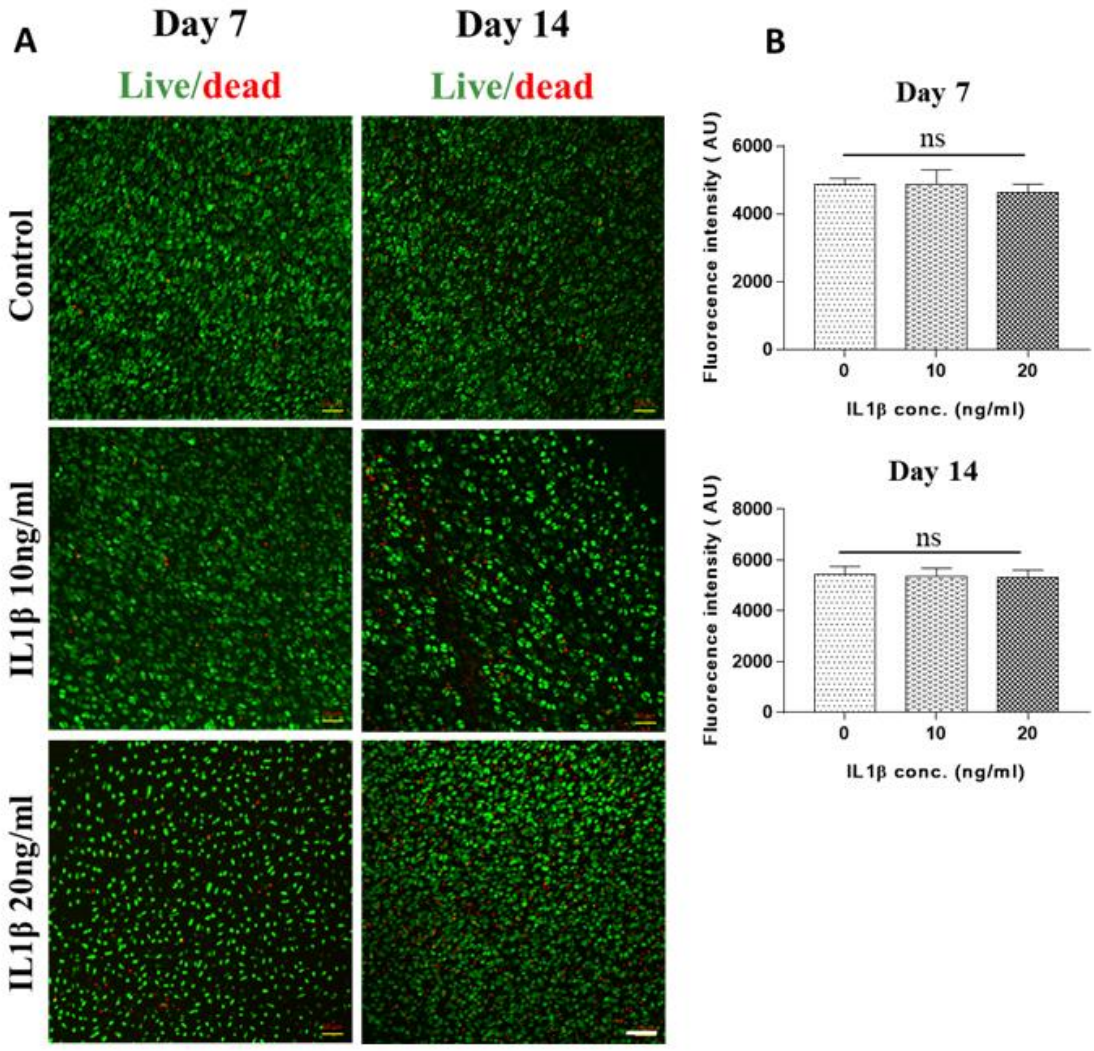

Fig. S2: Live/dead staining and metabolic activity of chondrocytes in goat cartilage explants
(A) Staining for live and dead chondrocytes with fluorescein diacetate (green) and propidium iodide (red) in goat cartilage explants after 7 and 14 days of treatment with IL1β respectively (scale bar – 50 µm). (B) Resazurin assay for metabolic activity of chondrocytes in goat cartilage explants after 7 and 14 days of IL1β treatment. ns, not significant (one-way ANOVA with Tukey's correction for multiple comparisons).

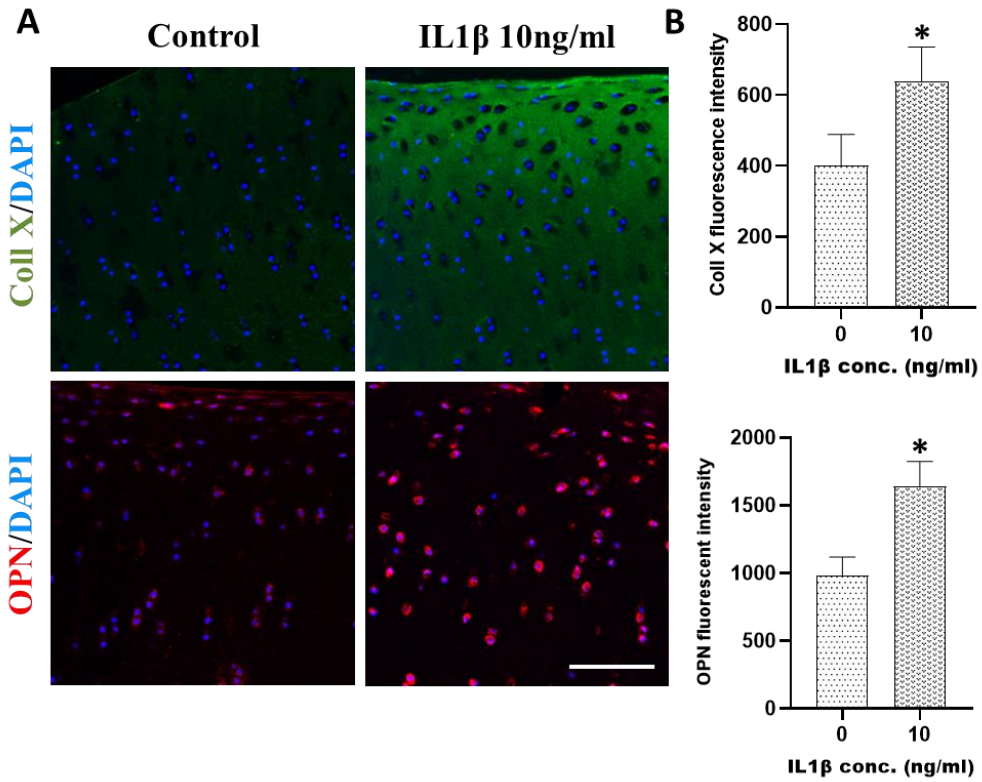

Fig. S3: Effect of IL1β treatment on chondrocyte hypertrophic markers
(A) Immunofluorescence micrographs of goat cartilage explant sections stained for collagen type X (Coll X) (green), osteopontin (OPN) (red), and nuclei (blue) (scale bar – 100 µm). (B) Fluorescence intensity quantification using image-based analysis. *, $p < 0.05$ with respect to control cartilage explants, n = 4 (one-way ANOVA with Tukey's correction for multiple comparisons).

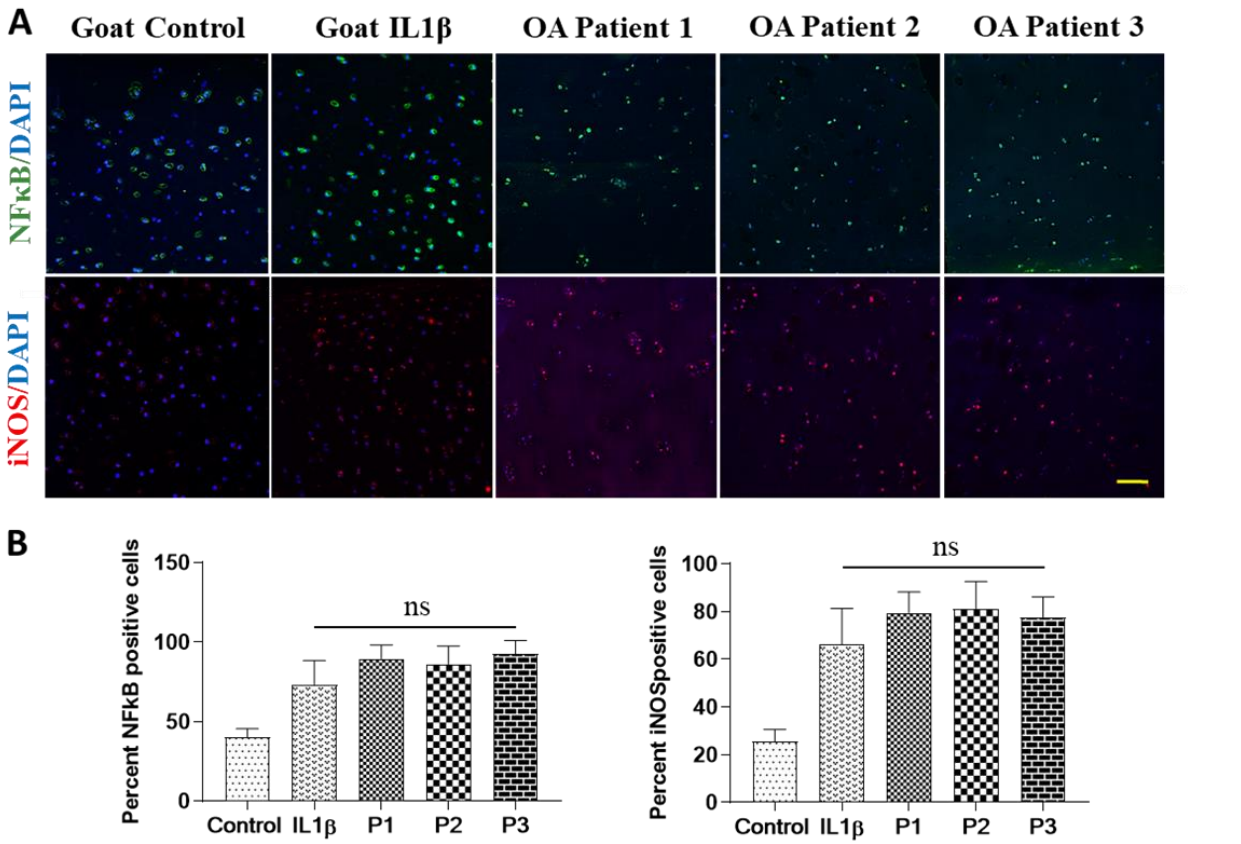

Fig. S4: Comparison of inflammatory marker expression in goat *ex vivo* OA model with human OA cartilage
(A) Immunofluorescence micrographs of goat and human cartilage sections stained for NFκB (green) and nuclei (blue); and iNOS (red) and nuclei (blue) (scale bar – 100 µm). (B) Image analysis-based quantification of % NFKB and iNOS positive cells. ns, no significant difference between the groups (one-way ANOVA with Tukey's correction for multiple comparisons).

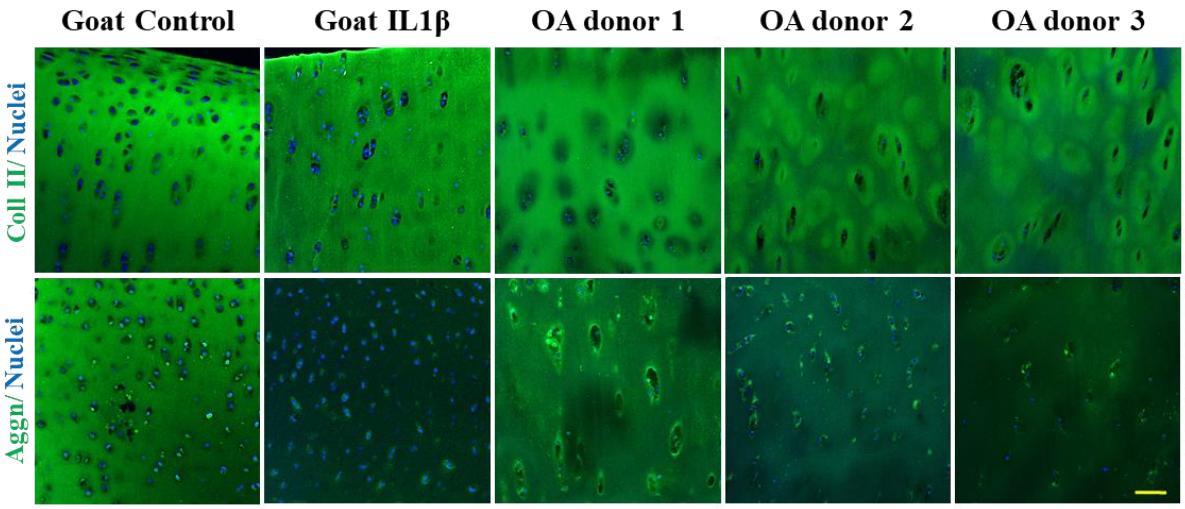

Fig. S5: Comparison of goat *ex vivo* OA model with human OA cartilage for collagen II and aggrecan expression
Immunofluorescence micrographs of goat cartilage explant sections stained for collagen type II (Coll II) (green), aggrecan (Aggn) (green), and nuclei (blue) (scale bar – 100 µm).

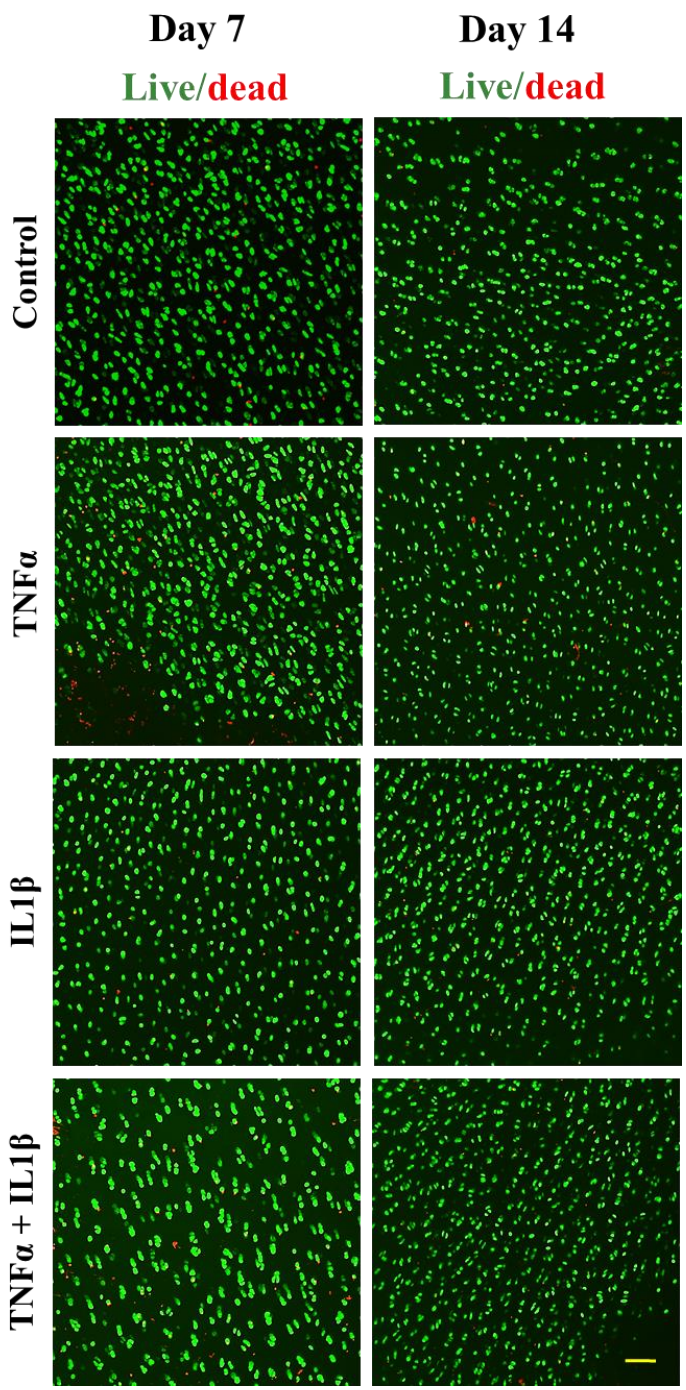

Fig. S6: Live/dead staining of chondrocytes in goat cartilage explants after treatment with cytokines (IL1β or TNFα or IL1β+TNFα)

Staining for live and dead chondrocytes with fluorescein diacetate (green) and propidium iodide (red) in goat cartilage explants after 7 and 14 days of treatment with different cytokines (scale bar – 50 µm).

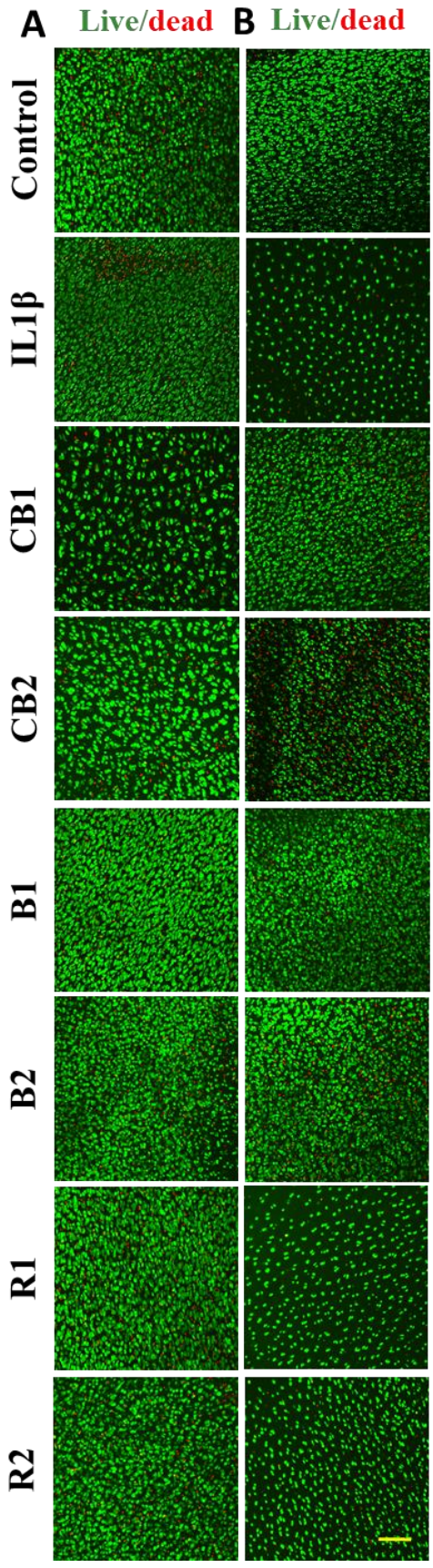

Fig. S7: Live/dead staining of chondrocytes in goat *ex vivo* OA model after treatment with celecoxib, BMP-7 or rapamycin

Staining for live and dead chondrocytes with fluorescein diacetate (green) and propidium iodide (red) in goat cartilage explants after (A) 7 days and (B) 14 days of treatment with different concentrations of celecoxib, BMP7 or rapamycin: CB1, celecoxib 0.1 µM; CB2, celecoxib 1 µM; B1, BMP7 50 ng/mL; B2, BMP7 100 ng/mL; R1, rapamycin 0.1 µM; R2, 1 µM. (scale bar – 100 µm).

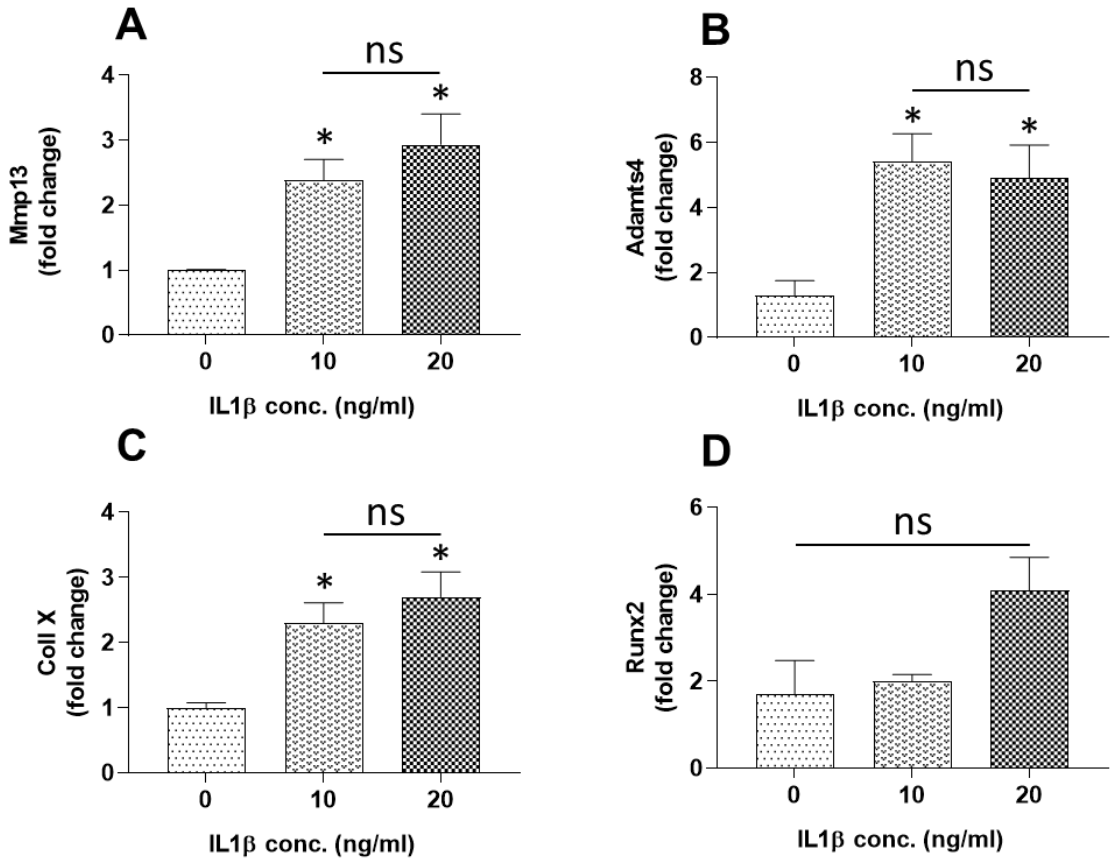

Fig. S8: Gene expression analysis for OA markers in goat *ex vivo* OA model
Quantification of mRNA expression for (A) *MMP13*, (B) *ADAMTS4*, (C) collagen type X (COL10A1) and (D) runt-related transcription factor 2 (*RUNX2*) normalized to *GAPDH* expression in goat cartilage explants after treatment with IL1β. *, $p < 0.05$ vs control cartilage explants; ns, not signficant, n = 6 (one-way ANOVA with Tukey's correction for multiple comparisons).

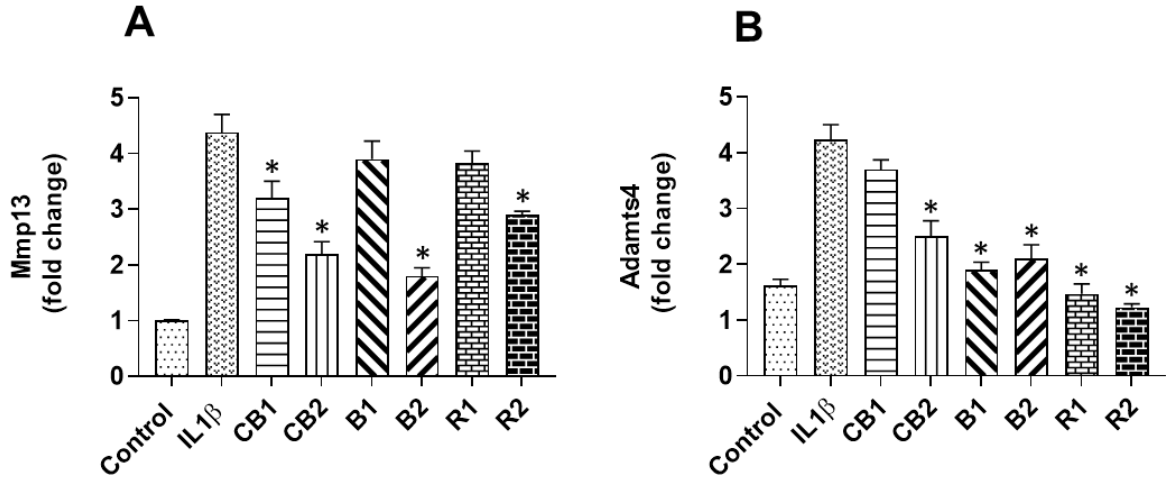

Fig. S9: Gene expression analysis of *ex vivo* OA model after treatment with celecoxib, BMP7 and rapamycin
Quantification of mRNA expression for (A) *MMP13* and (B) *ADAMTS4* (normalized to *GAPDH* expression) in goat cartilage explants after treatment with different concentrations of celecoxib, BMP7 and rapamycin. CB1, celecoxib 0.1 µM; CB2, celecoxib 1 µM; B1, BMP7 50 ng/mL; B2, BMP7 100 ng/mL; R1, rapamycin 0.1 µM; R2, 1 µM. *, $p < 0.05$ vs IL1β-treated cartilage explants, n = 6 (one-way ANOVA with Tukey's correction for multiple comparisons).