ARTICLE

# Supplementary Information - THE EFFECT OF CYCLIC FLUID PERFUSION ON THE PROINFLAMMATORY TISSUE ENVIRONMENT IN OSTEOARTHRITIS USING EQUINE JOINT-ON-A-CHIP MODELS

Johannes Heidenberger,[a†] Eva I. Reihs,[a,b] Jonathan Strauss,[a,b] Martin Frauenlob,[b] Sinan Gültekin[c], Iris Gerner[c], Stefan Tögel[a,d], Peter Ertl[b], Reinhard Windhager[a], Florien Jenner[c], Mario Rothbauer[a,b†*]

[a.] *Medizinische Universität Wien, Department of Orthopedics and Trauma Surgery, Karl Chiari Lab for Orthopaedic Biology , Währinger Gürtel 18-20, 1090 Vienna, Austria*
[b.] *Technische Universität Wien, Faculty of Technical Chemistry, Getreidemarkt 9/163, 1060 Vienna, Austria*
[c.] *University of Veterinary Medicine Vienna, Department for Small Animals and Horses, Centre for Equine Health and Research, Equine Surgery Unit, Veterinary Regenerative Medicine Laboratory, 1210 Vienna, Austria*
[d.] *Ludwig Boltzmann Institute for Arthritis and Rehabilitation, Vienna, Austria*
† Authors contributed equally.
*Correspondence: mario.rothbauer@tuwien.ac.at, mario.rothbauer@muv.ac.at
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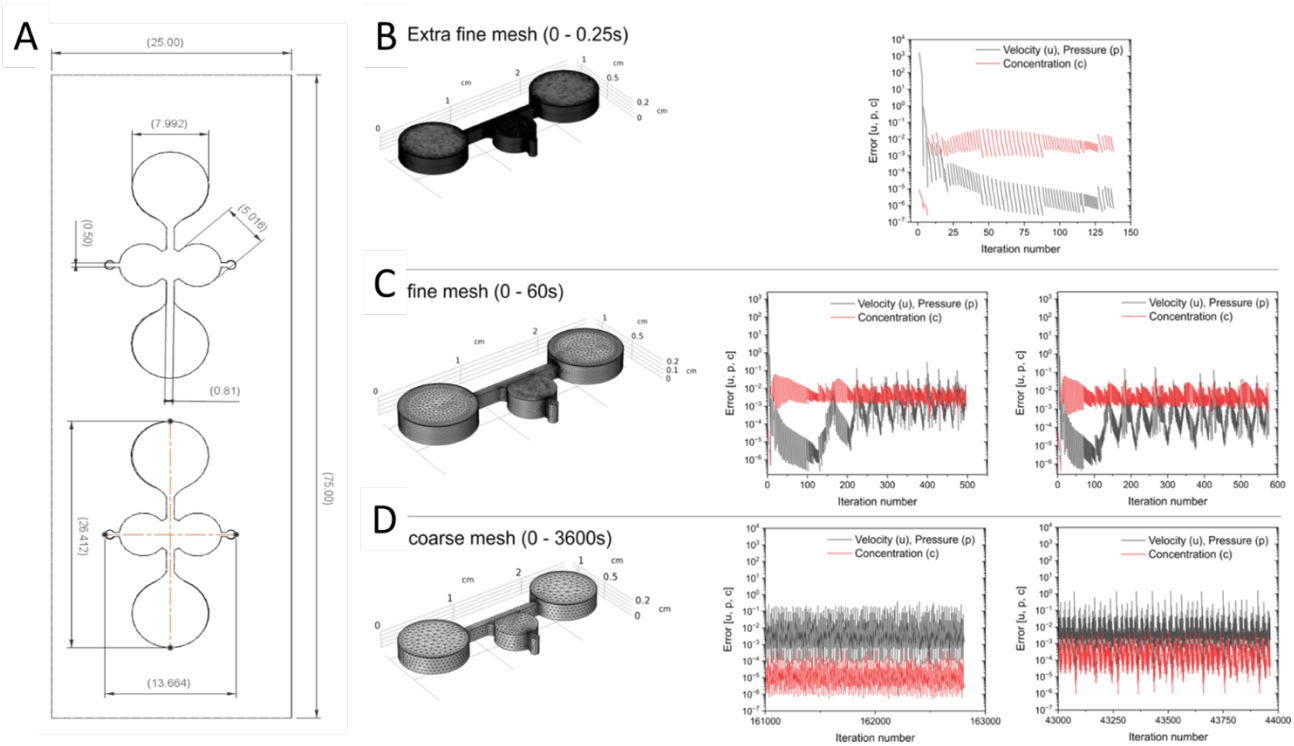
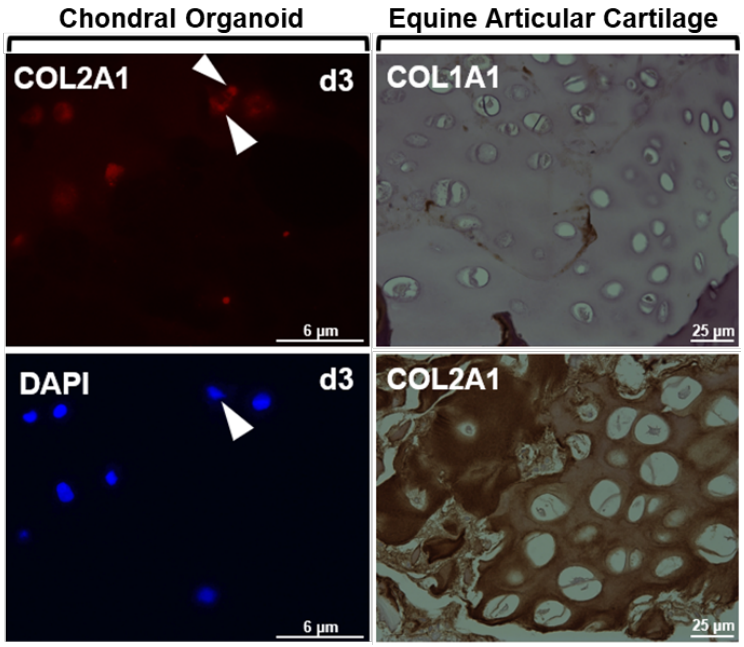
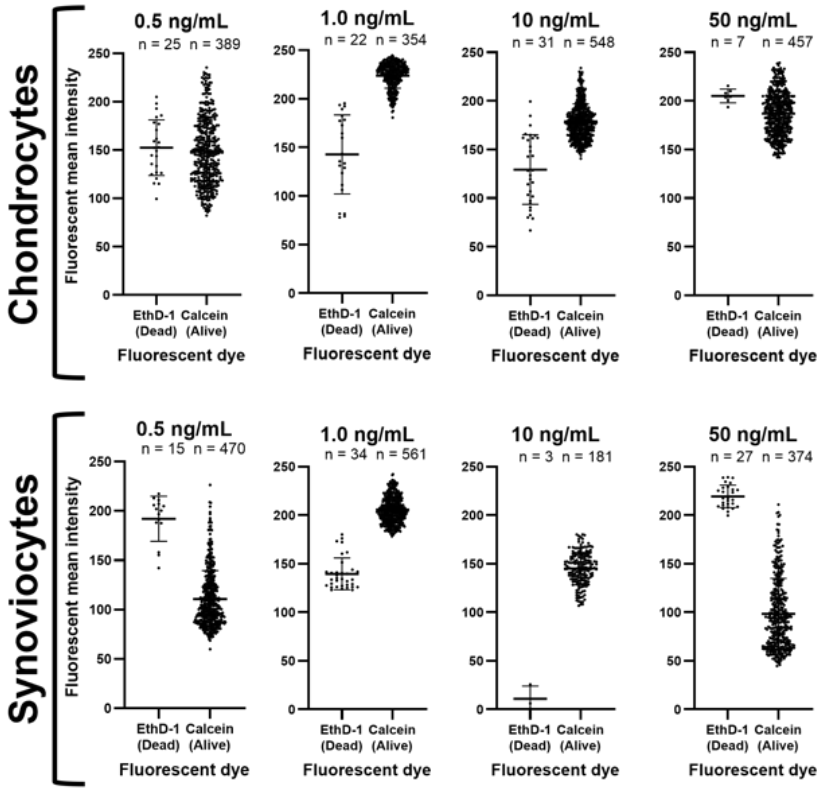
**Content:** SI Figure 1-7



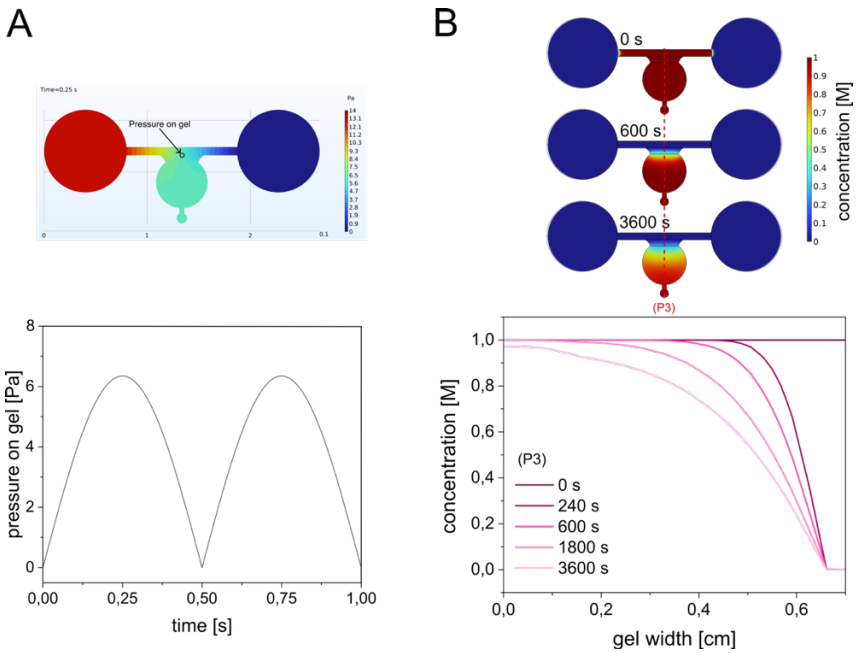

**SI Figure 1. (A) Design and (B-D) CFD meshing and convergence plots for the COMSOL CFD simulations. (Dimensions are in millimeters.)**



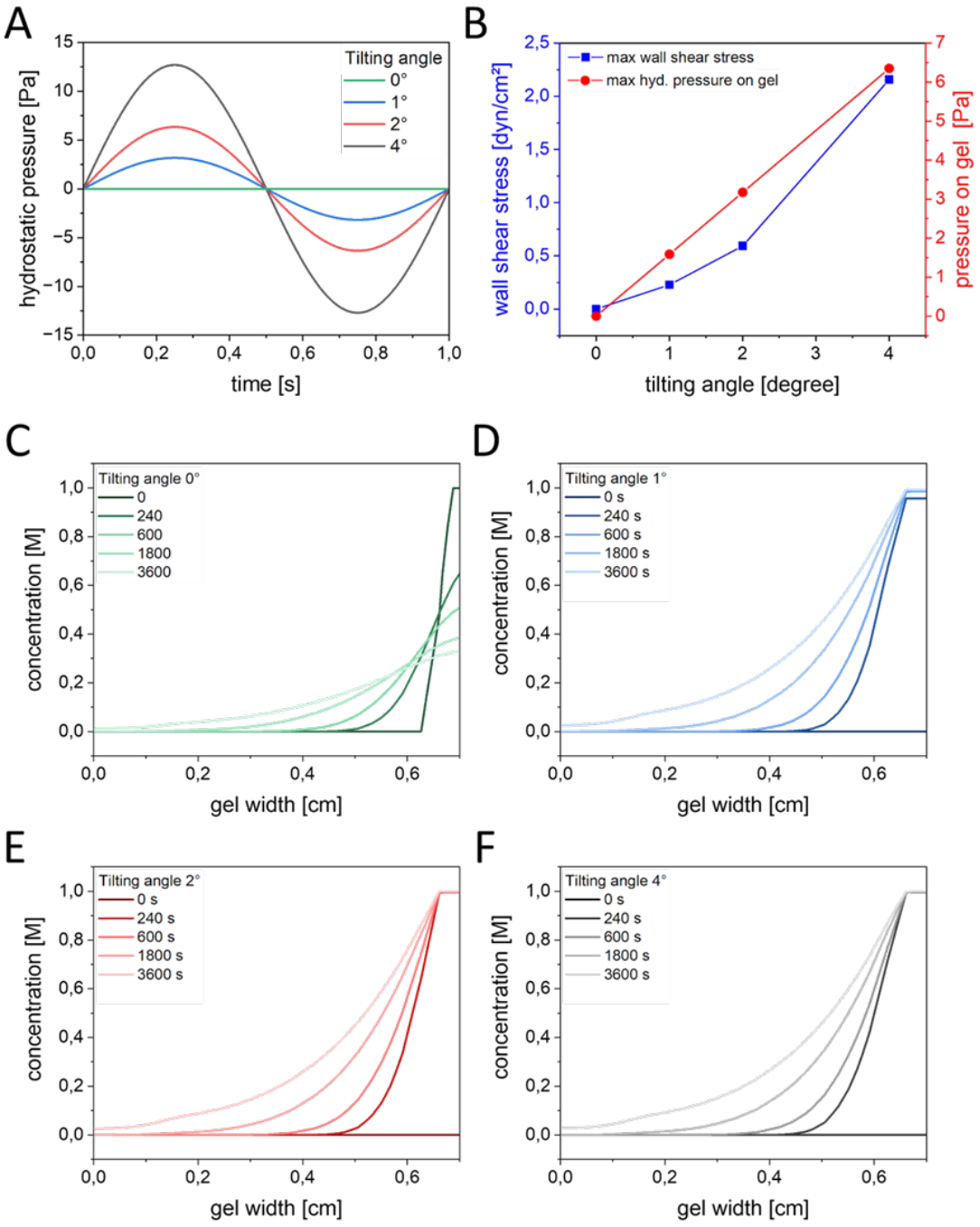

**SI Figure 2. Visual comparison of chondral organoids to real equine articular cartilage utilizing IF and IHC**. Immunofluorescence was used to stain cell nuclei and collagen type 2 in chondral organoids at day 3. Sections of equine articular cartilage were stained for collagen type 1 and 2 with immunohistochemistry. The white arrows highlight the position of the nucleus and the location of collagen type 2 expression in the chondral organoids.



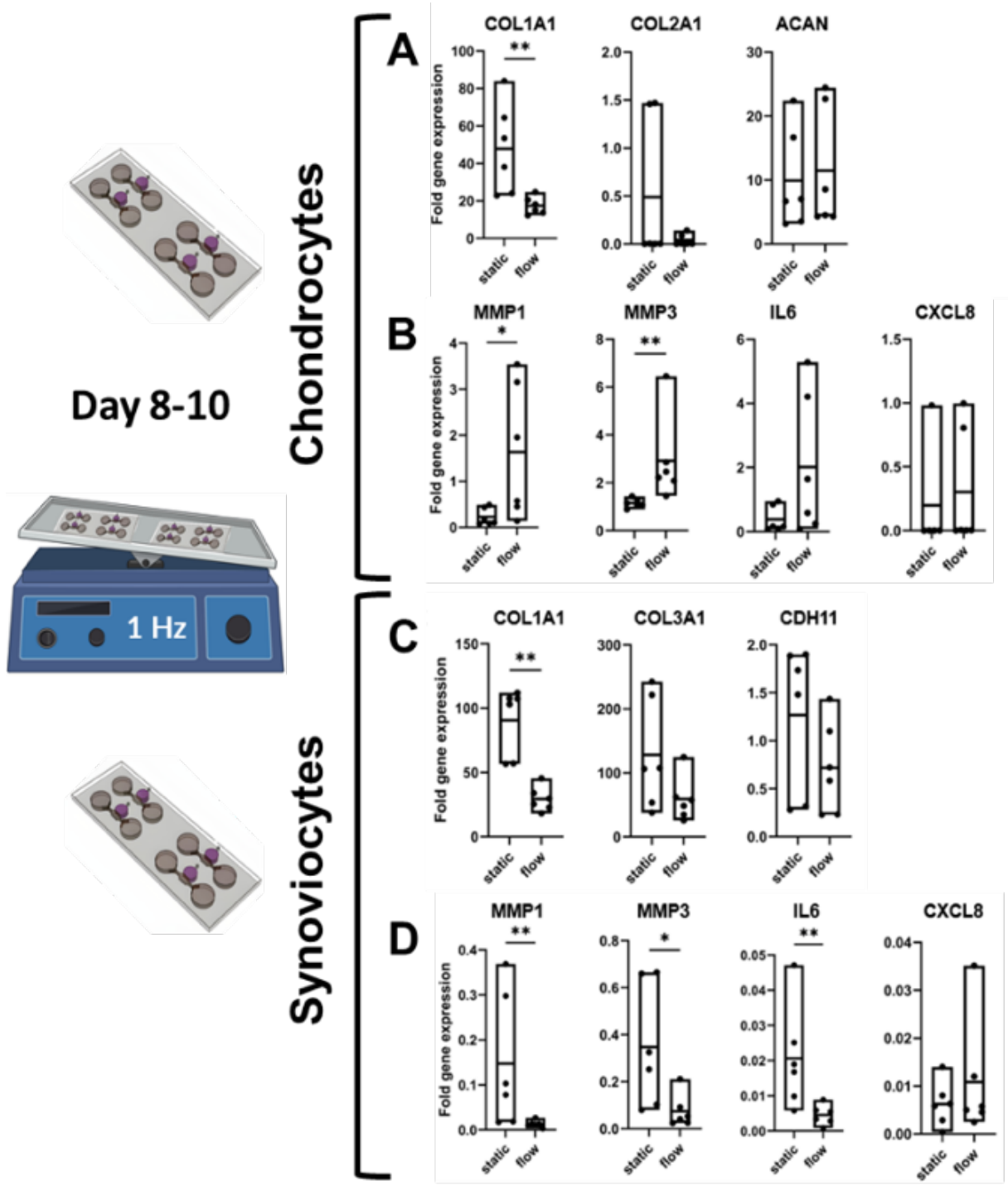

**SI Figure 3. Viability of chondrocytes and synoviocytes for different IL-1β concentrations.** Chondrocytes and synoviocytes were cultured for seven days in a 24-well plate with 0.5, 1.0, 10 and 50 ng/mL of IL-1β. Afterwards the cells were stained with calcein and propidium iodide to assess cell viability. Captured images were analyzed with ImageJ to count alive and dead cells and to determine the fluorescent intensity.



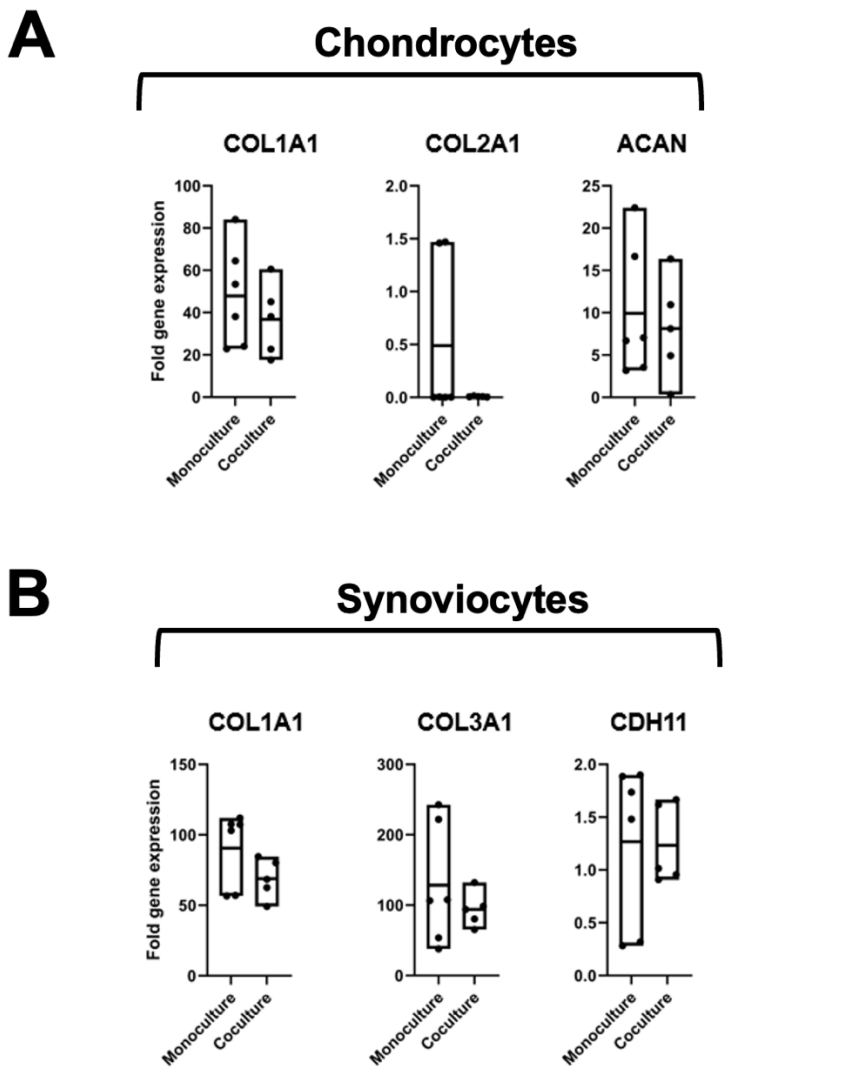

**SI Figure 4. CFD simulations of (A) pressure distributions and (B) molecule elution from the hydrogel compartment after 60 min of cyclic flow applications.**



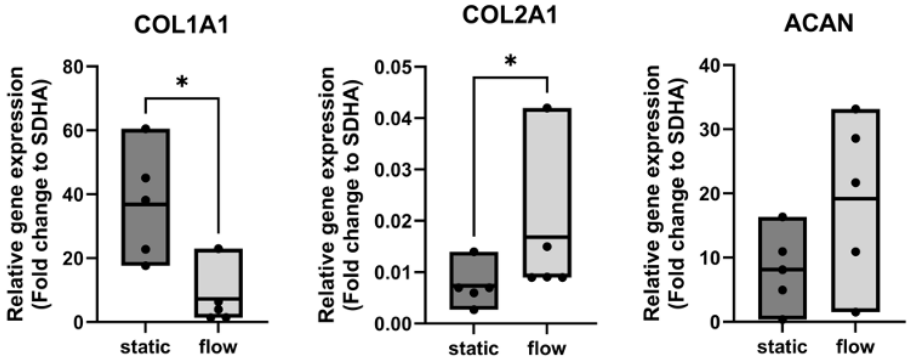

**SI Figure 5. Impact of tilting angle on mechanical forces and media supply.** The inlet function for various tilting angles (A) used in the CFD simulation to investigate the angles impact on maximal mechanical forces exerted on the gel in terms of wall shear stress and hydrostatic pressure (B) and media supply over time (C – F).



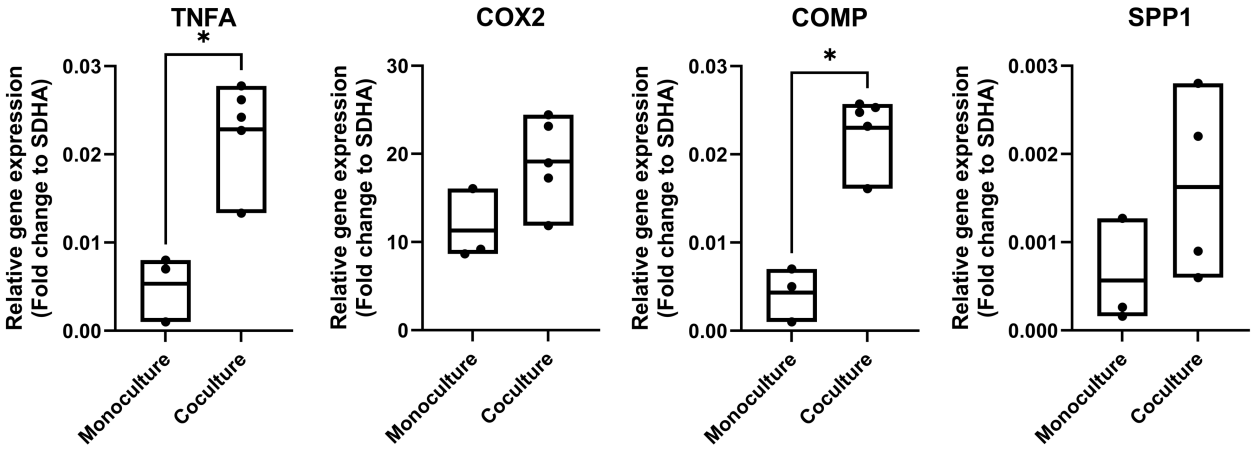

**SI Figure 6. Gene expression of untreated chondrocytes and synoviocytes in 3D monocultures under fluid flow.** Chondrocytes and synoviocytes were cultured for ten days in monoculture microchips. From day 7 to day 10 fluid flow was applied to one set of microchips while another one remained static. **(A)** Chondrocyte fold gene expression of matrix related genes: COL1A1, COL2A1, ACAN, **(B)** Chondrocyte fold gene expression of inflammation markers: MMP1, MMP3, IL6, CXCL8. **(C)** Synoviocyte fold gene expression of matrix related genes: COL1A1, COL3A1, CDH11. **(D)** Synoviocyte fold gene expression of inflammation markers. MMP1, MMP3, IL6, CXCL8. The data is expressed as mean fold change in reference to the housekeeping gene SDHA with n = 6 biological replicates (Mann-Whitney, *P < 0.05, **P < 0.01)




**SI Figure 7. Comparative PCR analysis of chondrocytes and synoviocytes cultivated in monoculture and coculture microchips for ten days**. (A) Chondrocyte and (B) synoviocyte gene expression of matrix related genes COL1A1, COL2A1, and ACAN, (B) Chondrocyte fold gene expression of inflammation markers: MMP1, MMP3, IL6, CXCL8. (C) Synoviocyte fold gene expression of matrix related genes: COL1A1, COL3A1, CDH11. (D) Synoviocyte fold gene expression of inflammation markers. MMP1, MMP3, IL6, CXCL8. The data is expressed as mean fold change in reference to the housekeeping gene SDHA with n = 3 for monoculture (duplicates) and n = 5 biological replicates for coculture. (Mann-Whitney, *P < 0.05, **P < 0.01)




**SI Figure 8. Comparative PCR analysis of the effect of fluid flow on chondrocyte cocultures with synovial organoids at day ten post-seeding.** Chondral organoid gene expression of matrix-related genes COL1A1, COL2A1, and ACAN for fluid actuated versus static control cocultures. The data is expressed as mean fold change in reference to the housekeeping gene SDHA with n = 5 biological replicates. (Mann-Whitney, *P < 0.05, **P < 0.01)




**SI Figure 9. Comparative PCR analysis of the effect of fluid flow of inflamed synovial organoids at day ten post-seeding in the presence of 10 ng/mL of IL-1β.** Synovial organoid gene expression of inflammation-related genes, including TNFA, COX2, COMP and SPP1 for fluid-actuated monocultures and cocultures with chondrocyte-containing organoids. The data is expressed as mean fold change in reference to the housekeeping gene SDHA with n = 5 biological replicates. (Mann-Whitney, *P < 0.05, **P < 0.01)