Electronic supplementary material for:

NFκB inhibition to lift the mechano-competence of mesenchymal stromal cell-derived neocartilage toward articular chondrocyte levels

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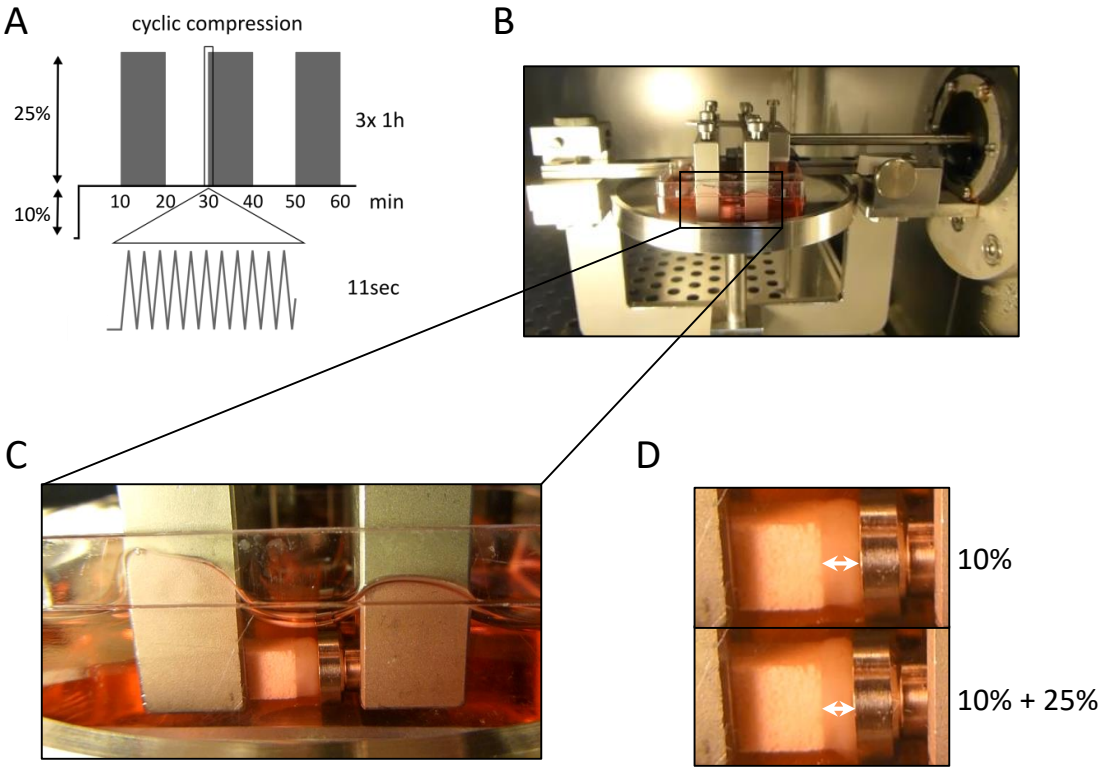
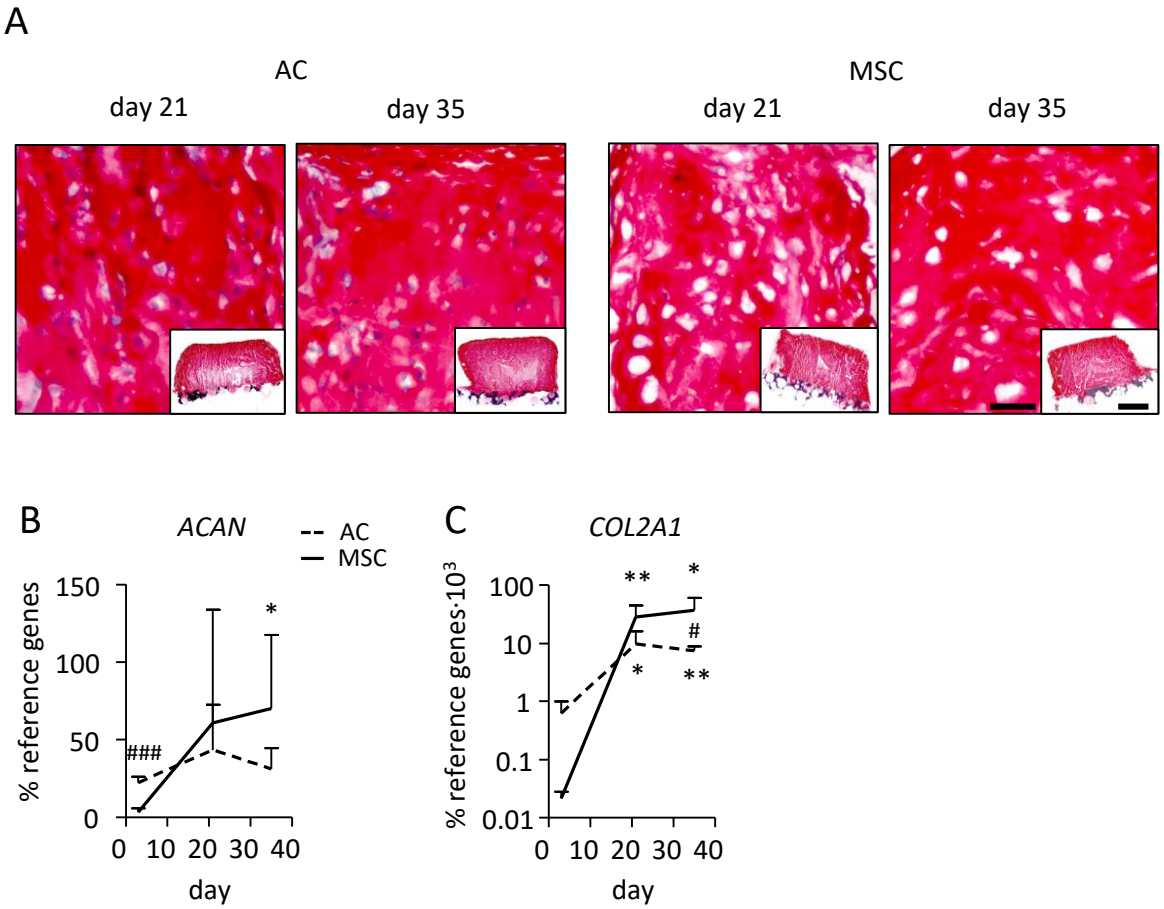
This pdf file includes:

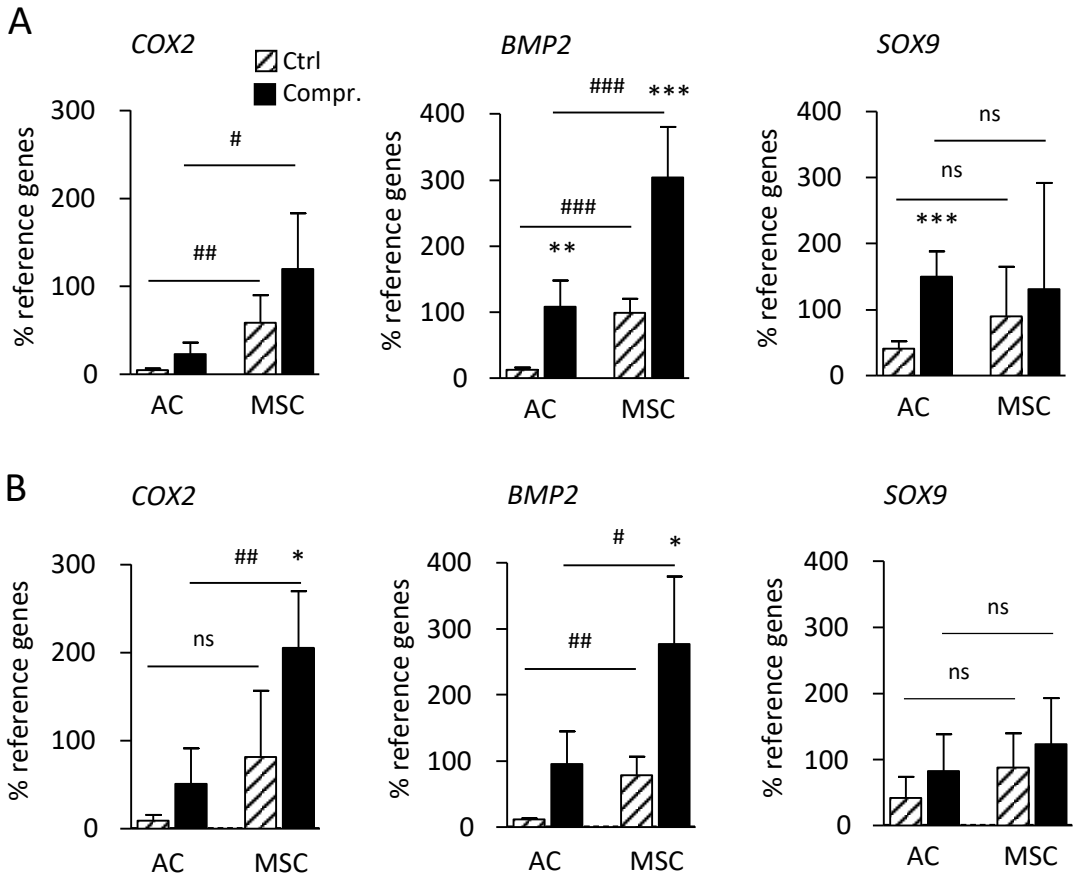
Supplementary Fig. S1-S4
Supplementary Table S1



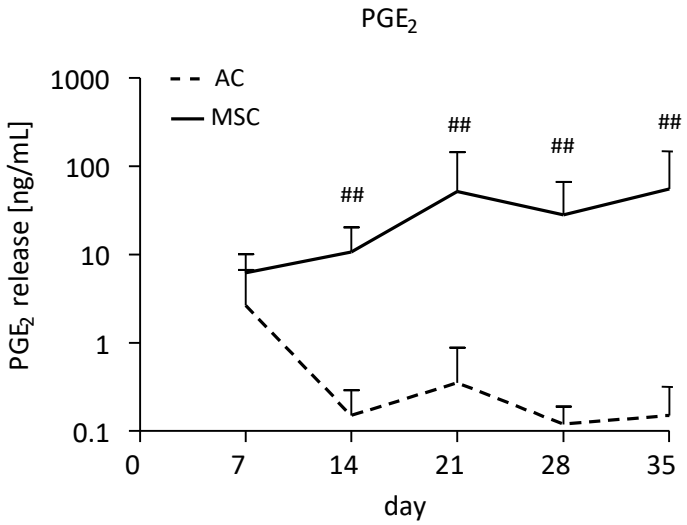
Supplementary Fig. S1. Loading conditions. AC- and MSC-seeded osteochondral units were subjected to a single 3-h cyclic compression episode in a custom-made bioreactor. **(A)** Schematic visualization of the applied loading protocol. **(B)** Representative picture of samples placed in the bioreactor. **(C)** Close-up picture of samples placed in the bioreactor. The piston touches the chondral phase of the osteochondral unit. **(D)** The 10% static offset and the superimposed additional 25% compression is shown for a representative sample.



Supplementary Fig. S2. Characterization of AC- and MSC-derived engineered cartilage. Biphasic osteochondral units were seeded with 5×10^5 human articular chondrocytes (AC) or mesenchymal stromal cells (MSC) and cultured for 3, 21 or 35 days under chondrogenic conditions. **(A)** Collagen type II was detected by immunohistochemistry of standard paraffin sections. Black: left-over β-TCP (scale bar: 50 µm (inset: 1 mm); representative pictures are shown; n=4-8 donors per group). **(B, C)** Mean gene expression ± SD is given as percentage of the mean levels of reference genes *HNRPH1* and *CPSF6* (n=3-8 donors). Two-tailed t-test with Bonferroni post-hoc test, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 in comparison to day 3, #p ≤ 0.05, ###p ≤ 0.001 AC vs. MSC at the same time point.



Supplementary Fig. S3. **Comparison of gene expression levels in AC- vs. MSC-derived cartilage.** RNA was isolated at termination of loading of compressed samples and free-swelling controls and gene-specific mRNA levels were determined by qPCR. **(A)** *COX2, BMP2* and *SOX9* expression levels in AC- and MSC-derived cartilage on day 21. **(B)** *COX2, BMP2* and *SOX9* expression levels in AC- and MSC-derived cartilage on day 35. Gene expression was normalized to reference genes *HNRPH1* and *CPSF6*. T-test with Bonferroni correction, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 compressed vs. free-swelling controls, #p ≤ 0.05, ##p ≤ 0.01, ###p ≤ 0.001 AC vs. MSC (n=4-8).



Supplementary Fig. S4. PGE_2 release from AC and MSC pellets cultured under chondrogenic conditions over time. Conditioned medium supernatants (2 days) from AC and MSC-derived chondrocyte pellets were collected weekly and analyzed for PGE_2 content by ELISA (n=6 donors). Mann-Whitney U test, ##p ≤ 0.01 AC vs. MSC at the same time point.

Supplementary Table S1. List of qRT-PCR primers used in this study

Gene Name	Sequence (5'-3')
ACAN	GCACATGCCTTCTGCTT GGAACCACTTGGGTCACG
BMP2	ACGAGGTCCTGAGCGAGTTC GAAGCTCTGCTGAGGTGATAA
BMP6	ATTACAACAGCAGTGAATTGA TTCATGTGTGCGTTGAGTG
COL2A1	TGGCCTGAGACAGCATGAC AGTGTTGGGAGCCAGATTGT
COX2	TTCAAATGAGATTGTGGAAAAATTGCT AGATCATCTCTGCCTGAGTATCTT
CPSF6	AAGATTGCCTTCATGGAATTGAG TCGTGATCTACTATGGTCCCTCTCT
DUSP5	CTCCCACTTTCAAGAAGCAA GGCAGGATCTCAGATTCGTA
FOS	TCCAGTGCCAACTTCATTCC GCTGCAGCCATCTTATTCCT
FOSB	CCAGGGAAATGTTTCAGGCT GAAGAGATGAGGGTGGGTTG
HNRPH1	GATGTAGCAAGGAAGAAATTGTTCAG CACCGGCAATGTTATCCCAT
SOX9	GTACCCGCACTTGCACAAC TCGCTCTCGTTCAGAAGTCTC