Supplementary figures and tables

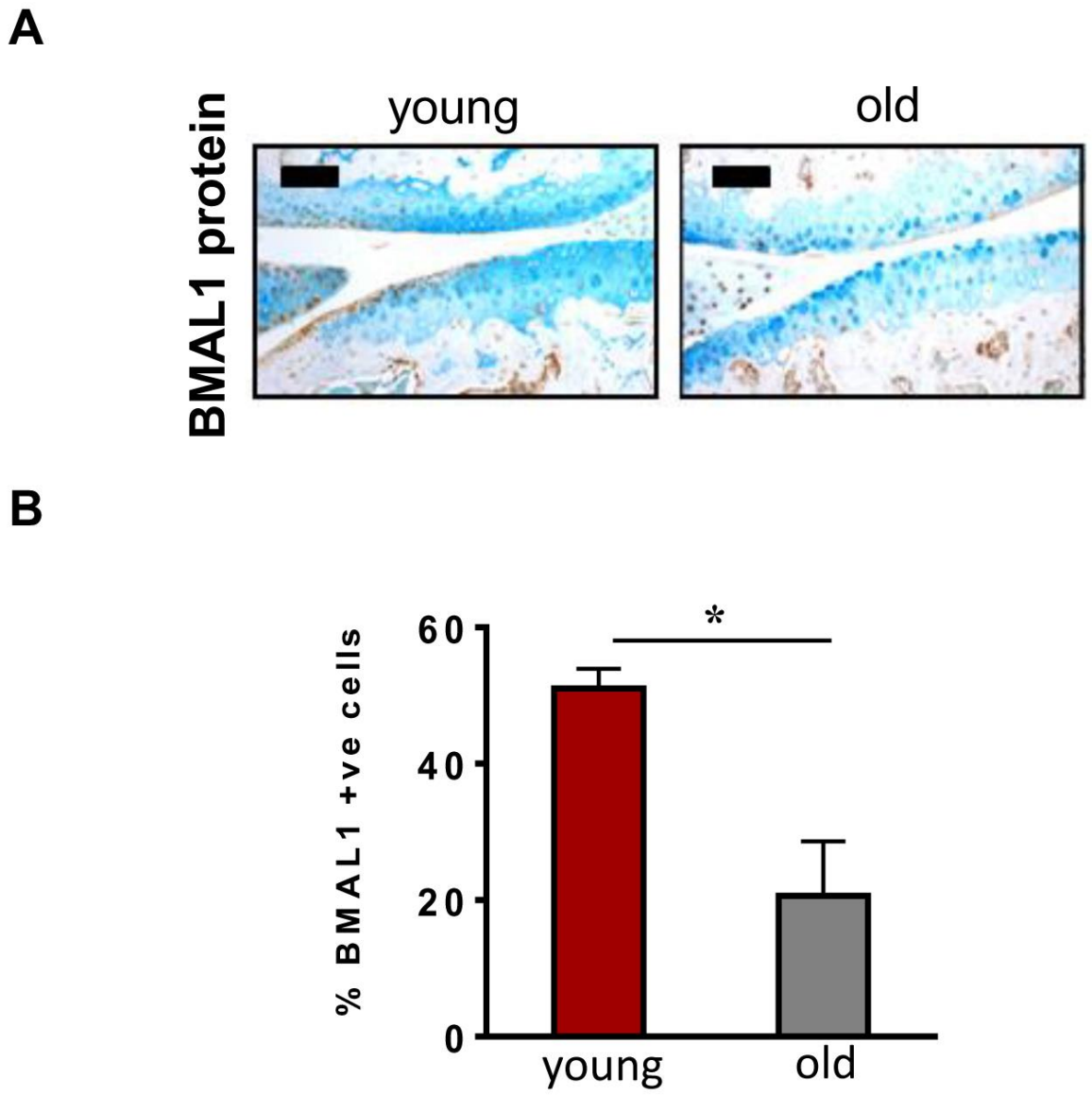


Figure S1, Reduction of BMAL1 positive cells in mouse knee joint articular cartilage with aging.

(A) DAB immunohistochemistry against BMAL1 protein (brown), counterstained using methyl green nuclear stain (blue/green), showing profound reduction in aged cartilage, representative n=3.

(B) Histograms (mean ± SEM) quantifying staining in (A), showing reduced BMAL1 staining. Data are expressed as % of chondrocytes positively stained, t test showed significant effect of age (*, $p<0.05$).

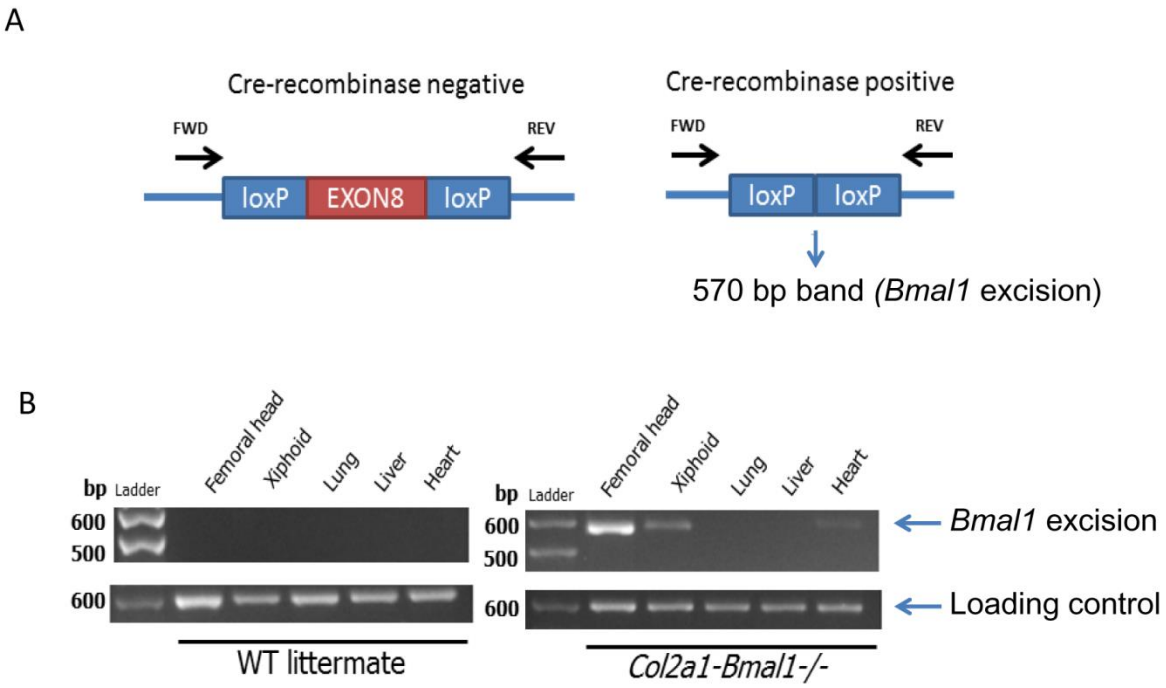


Figure S2, Genotyping confirmation for the deletion of the *ΔBmal1* allele in cartilage.

(A) Genotyping strategy for detection of the *ΔBmal1* allele in which exon 8 is deleted (adapted from[21]).

(B) Genotyping of DNA from multiple peripheral tissues of Cre negative control mice (WT, left) and Cre positive *Col2a1-Bmal1*$^{-/-}$ (right) littermates. Upper panel shows a 570 bp band indicating presence of the *ΔBmal1* deletion; lower panel shows 600 bp band indicating *PER2::luc* expression as a loading control.

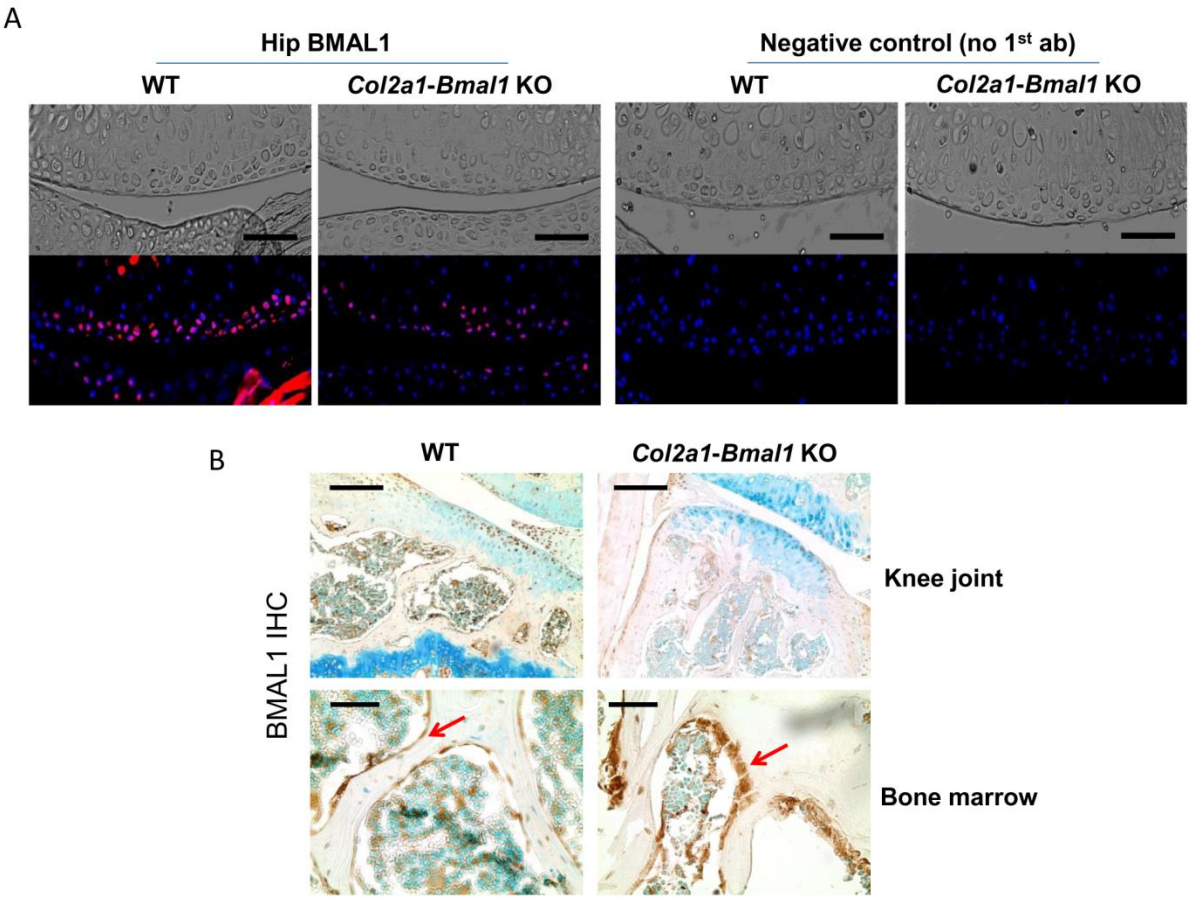


Figure S3, Reduced BMAL1 expression in chondrocytes of mouse hip cartilage (A), but not in osteoblasts (red arrow) of mouse knee joint. Negative controls (without primary antibody) were included on the right panels in (A). Scale bar = 50 μM.

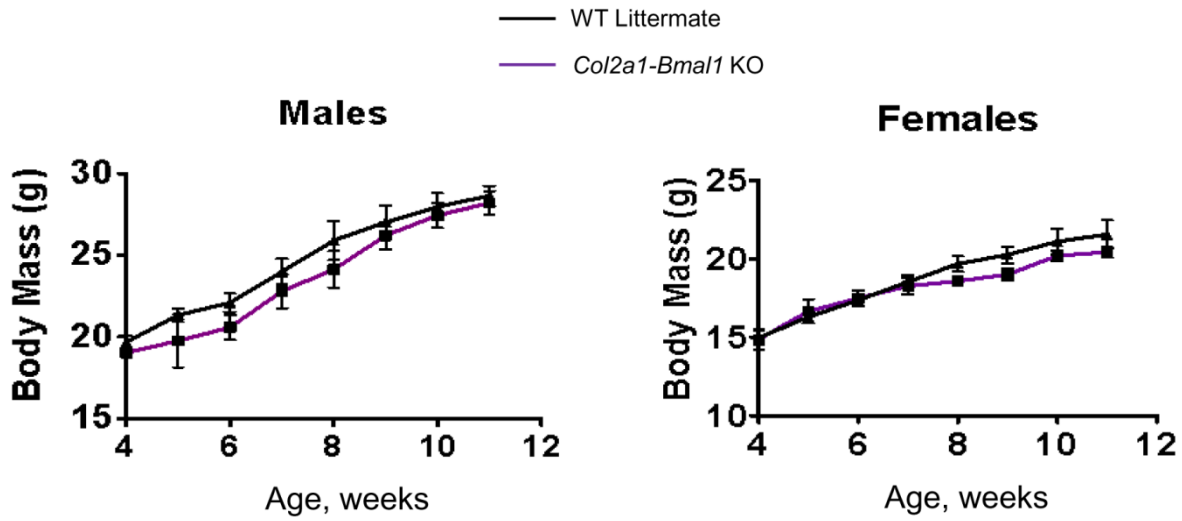


Figure S4, *Col2a1-Bmal1*$^{-/-}$ mice had normal growth rate and body weight.

Mice were weighed weekly, n=8, mean ± SEM, two way repeated measures ANOVA, no significant effect of genotype ($p > 0.05$) for either male or female animals.

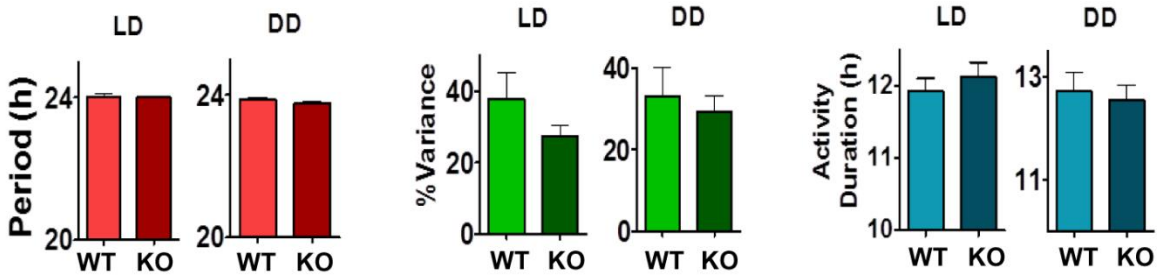


Figure S5, Normal rhythmic wheel-running behaviour of the *Col2a1-Bmal1*$^{-/-}$ mice. Quantification of period, % variance measure of rhythm strength, and duration of active phase of animals in LD and DD. Mean ± SEM. T-test, $p>0.05$ for all three measurements.

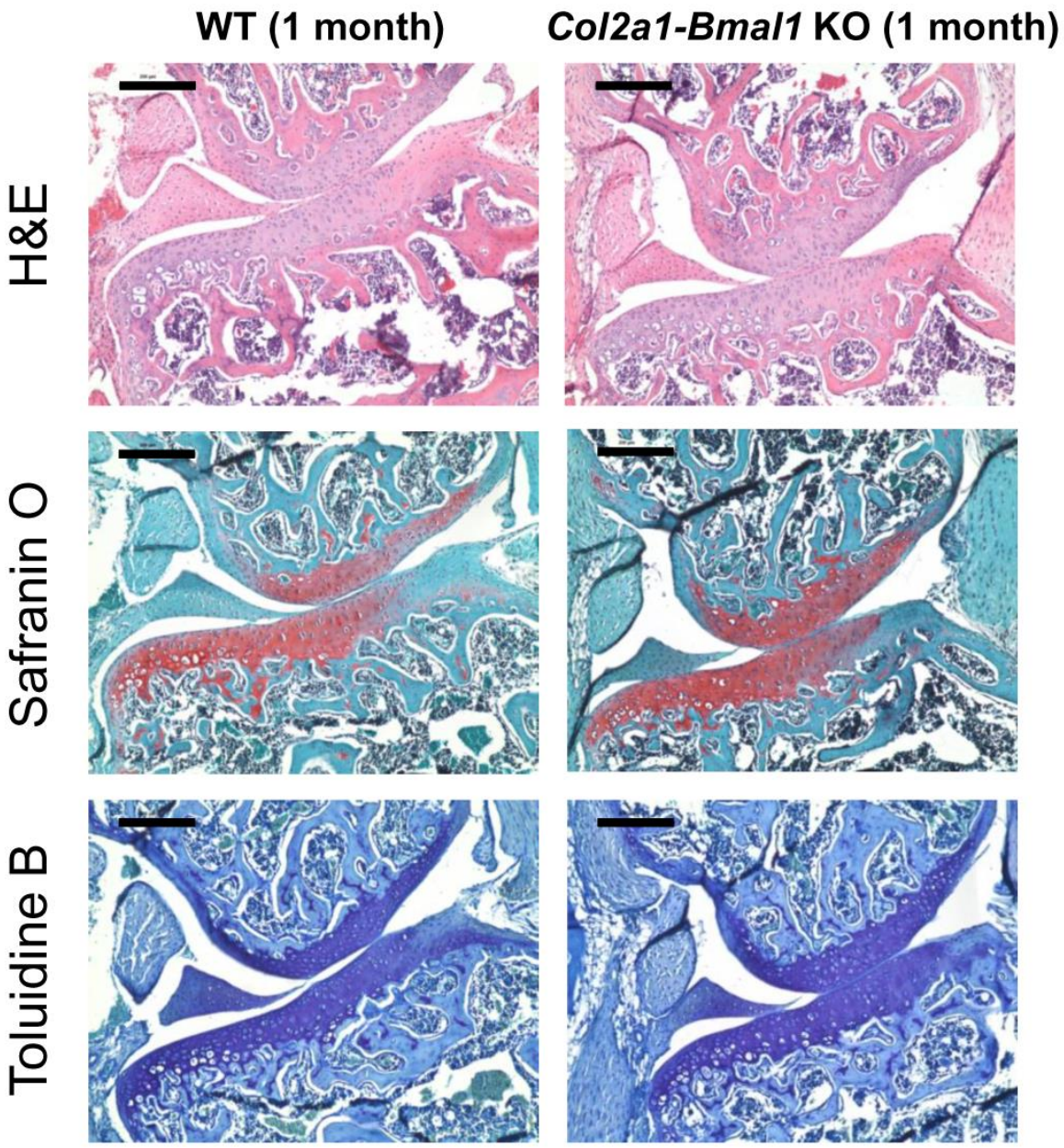


Figure S6, No discernible defects in knee cartilage of 1 month old KOs.

H&E, Safranin O/Fast Green/Hematoxylin stain (red - cartilage, blue - cytoplasm, black - nuclei) and Toluidine blue stain (blue - proteoglycan) of frontally embedded knee joint articular cartilage of 1 month old *Col2a1-Bmal1*$^{-/-}$ mice and WT littermate (representative images, n=6), scale bar = 200 µm.

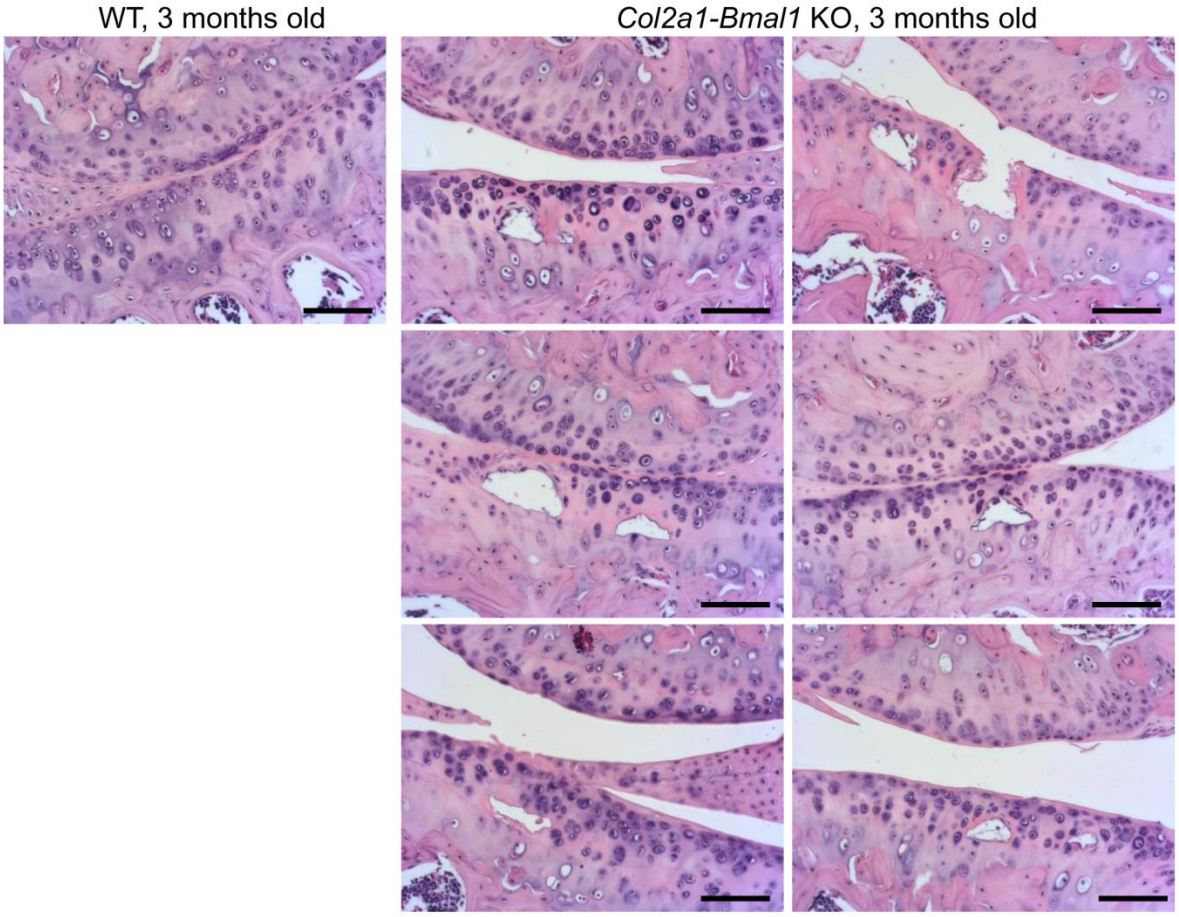


Figure S7, The deep zone cartilage lesions in individual *Col2a1-Bmal1*$^{-/-}$ animals. H&E images of further independent experiments were shown. Lesions were never seen in wild type animals. Scale bar = 100 µM.

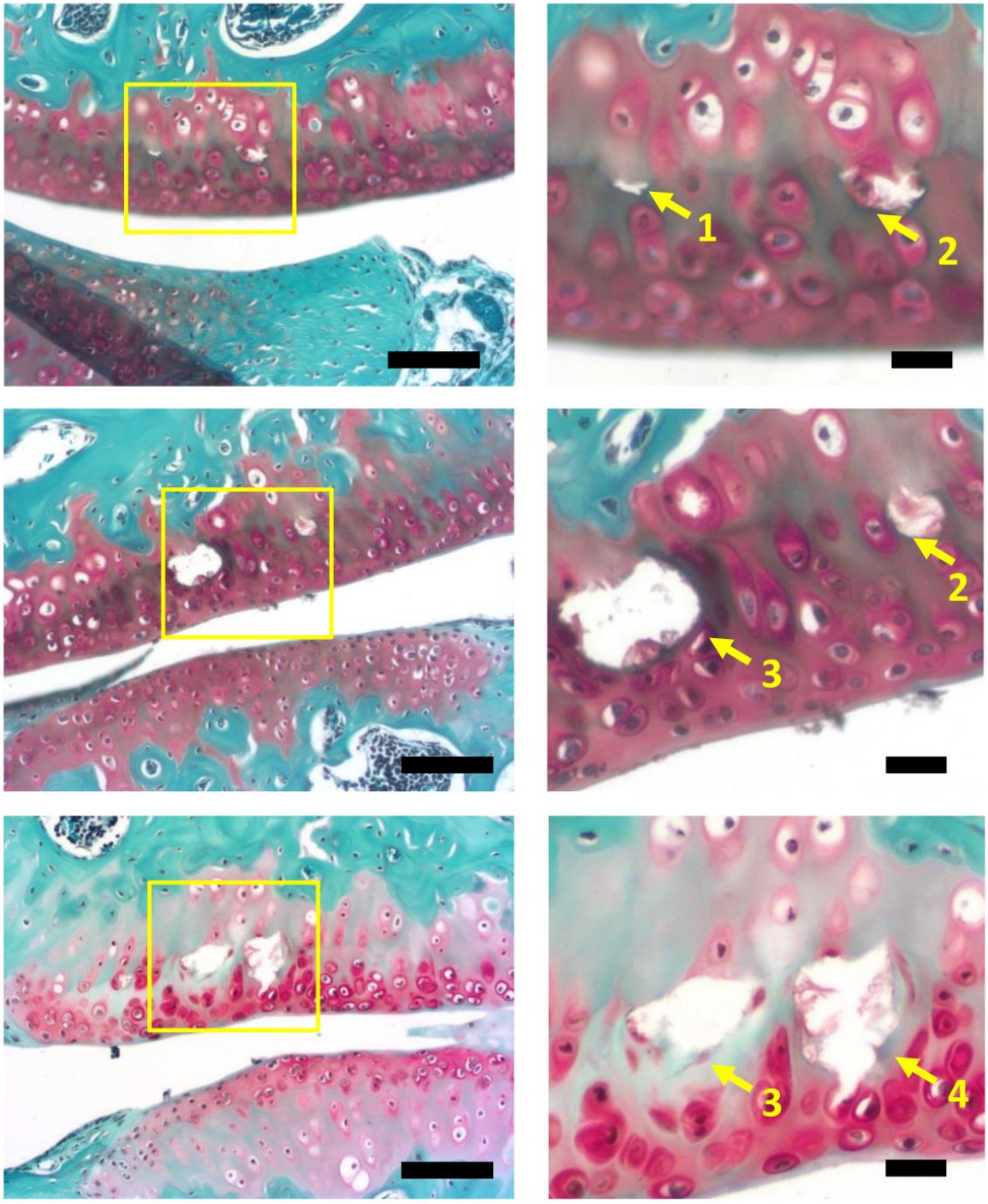


Figure S8, Further characterization of lesions occurring in cKO articular cartilage. The lesions were categorised according to the height of the lesion as a percentage of the height of uncalcified cartilage measured from the tidemark. Category 1: <10%, Category 2: 11-30%, Category 3: 31-50%, Category 4: >50%, including surface breach. Scale bar = 100 µm on left panels, 25 µm on enlarged panels on the right.

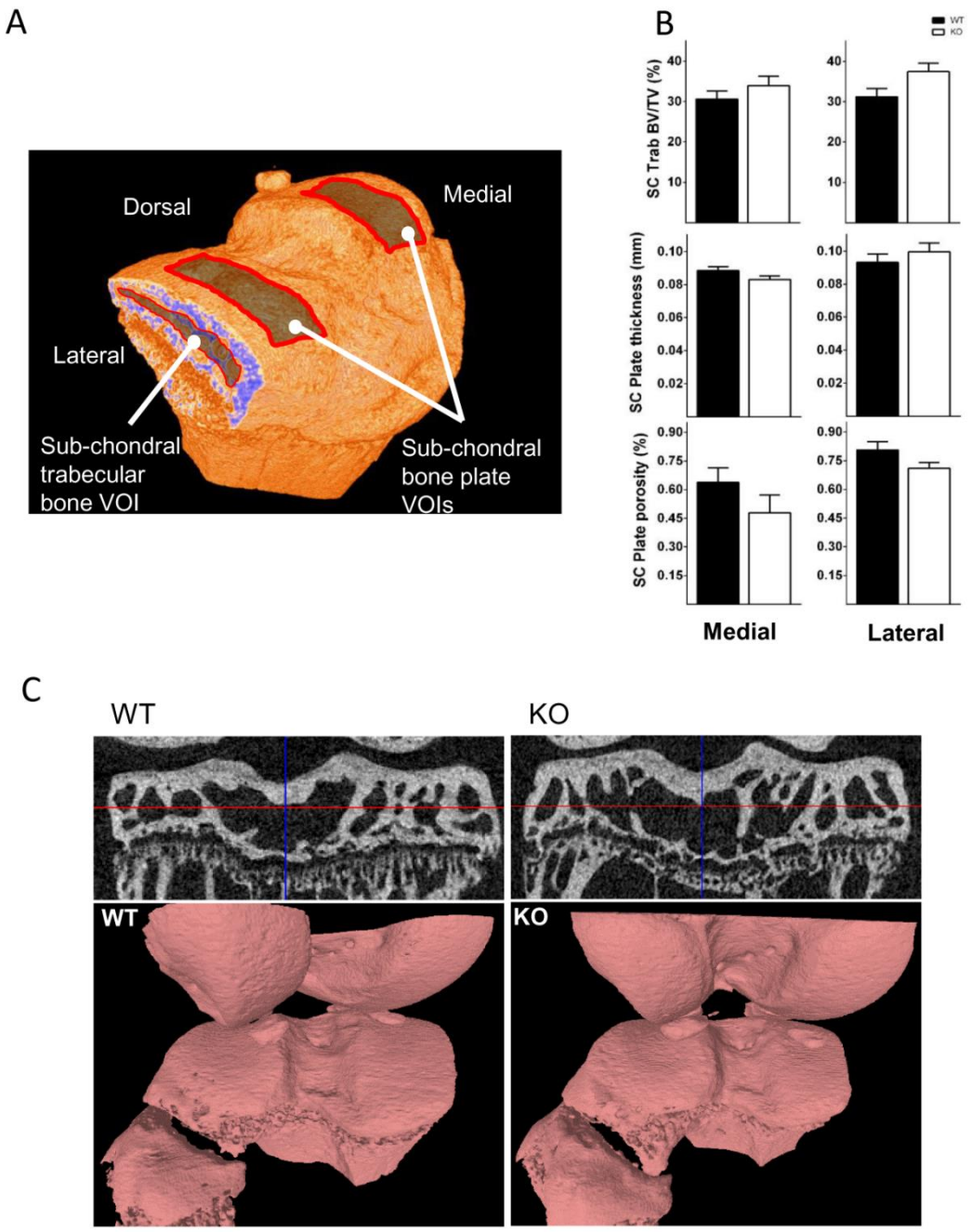


Figure S9, X-ray micro-CT analysis of the tibia sub-chondral bone in *Col2a1-Bmal1*$^{-/-}$ mice. (A) A 3-dimensional model of the proximal tibia of 3-4 months old female mice is shown, with 2 volumes of interest (VOIs; the medial side and the lateral side) chosen for analysis. These VOIs included the subchondral (SC) bone plate and subchondral trabeculae. The subchondral trabecular bone VOI were performed 0.3 mm above the growth plate. Regions of interest were hand-drawn around the trabecular bone in the medial and lateral sides. Analysis was performed on a 0.1 mm height. Selection of the sub-chondral bone plate VOI was performed on a region of 0.5mm medio-lateral width and 1mm dorso-ventral length on the medial and lateral sides of the load-bearing areas of the tibia plateau. (B) X-ray microCT

analysis of the subchondral trabecular bone volume fraction (%BV/TV) and bone plate thickness and porosity (n=4). (C) A representative example of coronal section of tibia subchondral bone (top images) and 3-dimensional model of the femoral tibia joint (bottom images) in WT and KO mice. Data shown as Mean ± SEM. Statistical analysis performed using unpaired T-test.

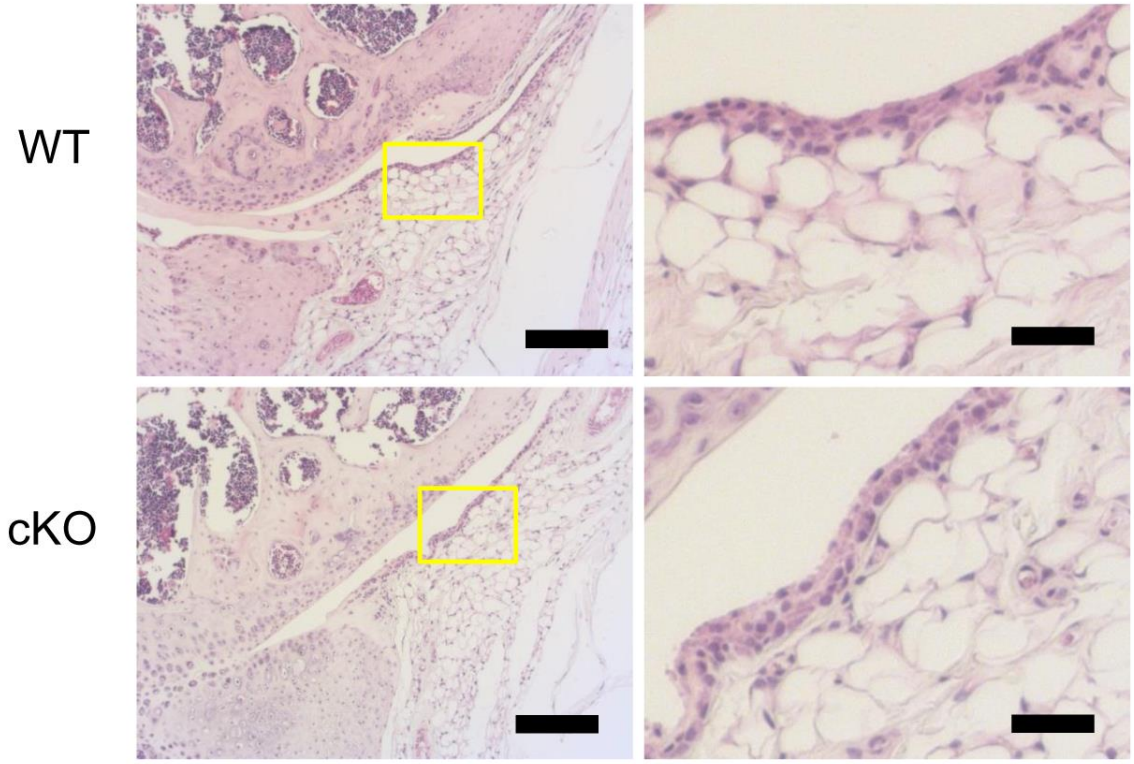


Figure S10, Normal synovium in the knees of *Col2a1-Bmal1*$^{-/-}$ mice. There were no signs of thickening or infiltration of neutrophils.

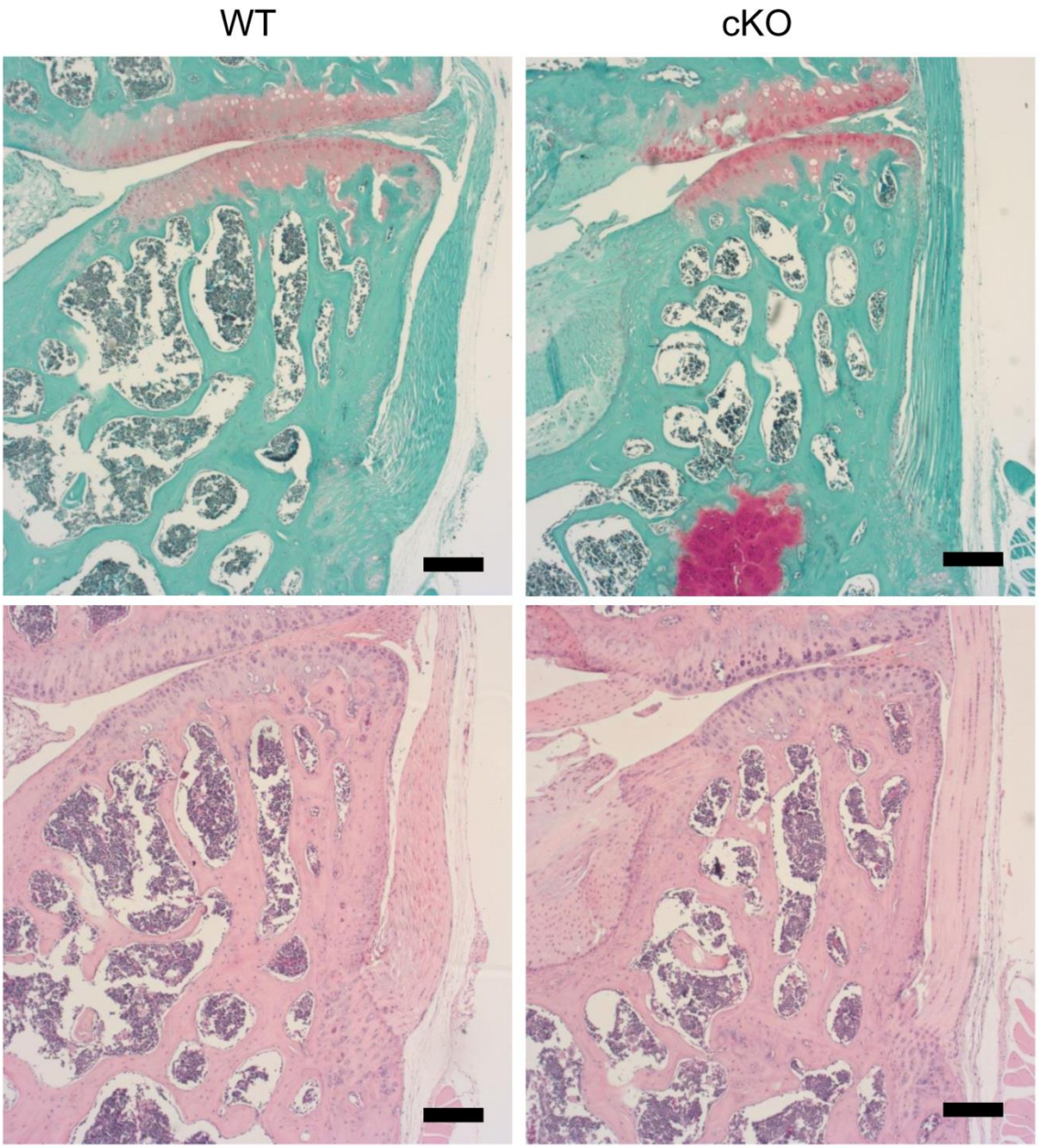


Figure S11, Normal ligaments in the knees of *Col2a1-Bmal1*$^{-/-}$ mice. There were no signs of degeneration.

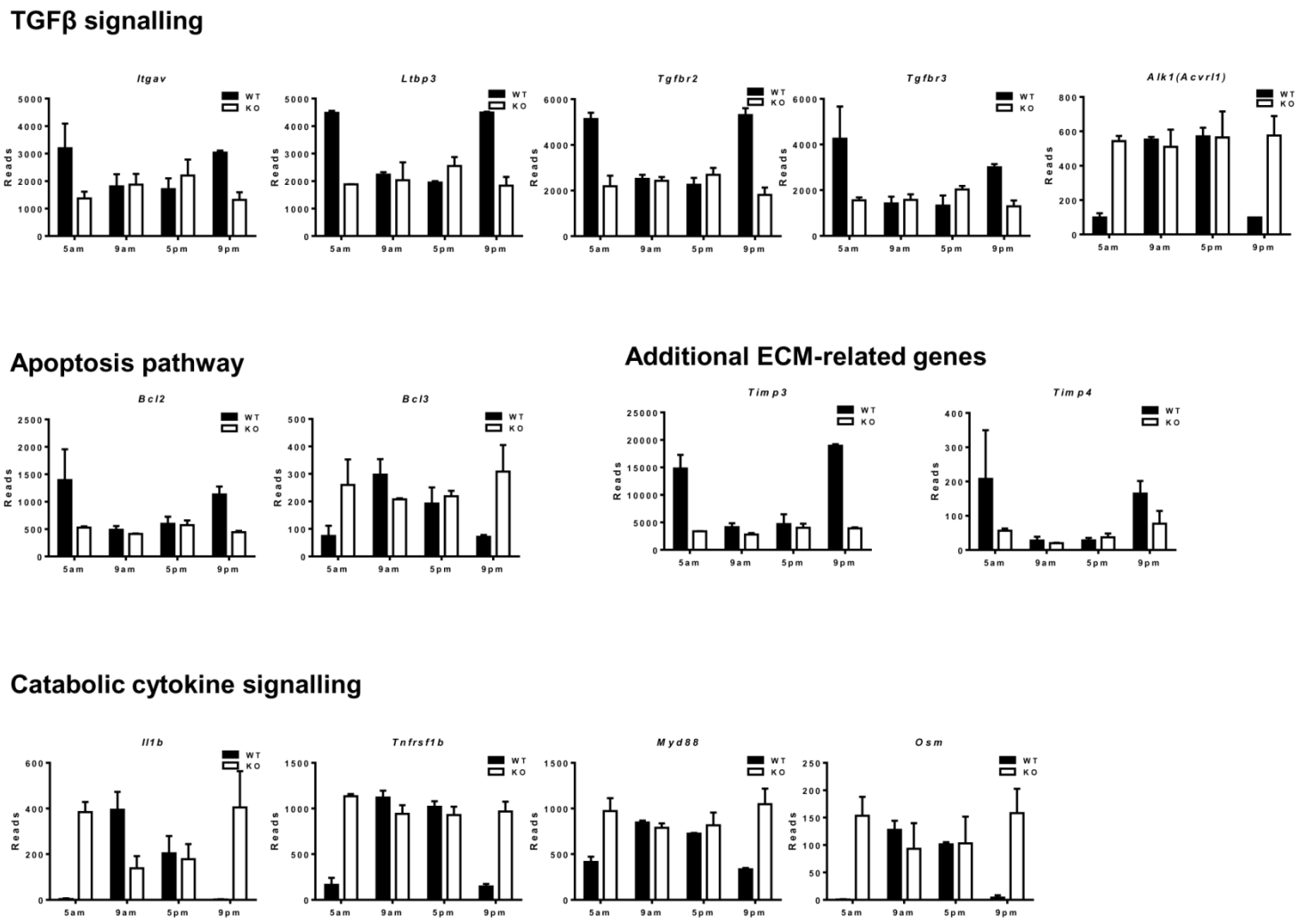
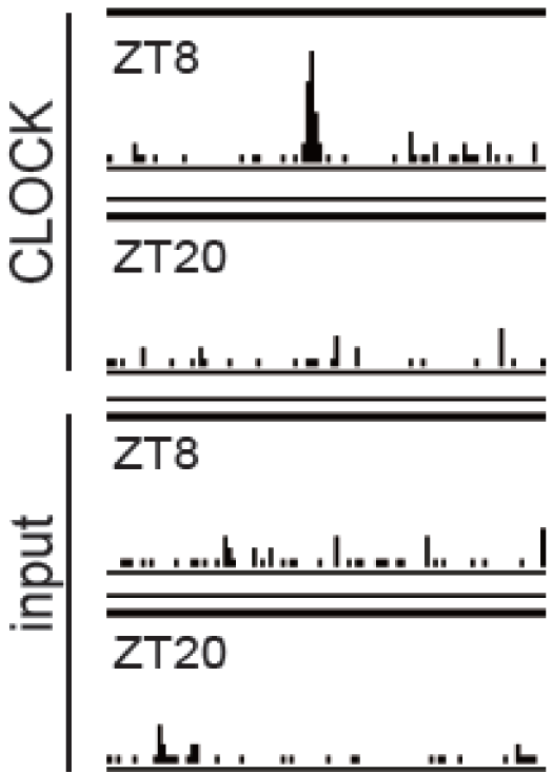


Figure S12, Plots of raw RNAseq data for a selection of genes relating to cartilage homeostasis/OA pathogenesis. For instance, consistent with the predicted inhibition of TGFβ signalling in cKO cartilage, significant down-regulation of key TGFβ signalling molecules or targets were observed (including *Itgav, Ltbp-3*, *Tgfβr2, Tgfβr3 and Alk1*).

A



B

±100 bp around CLOCK-binding site

AATCGTGACTTCTTTACCAATGTCCCCATTTAGCCACACA
CTCATCTCCTCACGTGGCTCACACAGCTGAGCCTGAGTTC
CACAGTCTCGGGGACCTCACACACCCTGTTGTGCTGACTC
TGAATGTTGATGGAACTTTCACCCTCAGCCACGACAGGGC
AGCTCATGATGCAATCTGCCTCATACCAAACAGAGCACTC

Figure S13. CLOCK protein rhythmically binds to a putative E-box sequence in intron 3 of the mouse *Nfatc2* gene in mouse liver.

(A) ChIPseq raw data[34] for the binding of CLOCK to this region. ZT, zeitgeber time. ZT8 = 8 hours after light on, ZT20 = 8 hours after light off. (B) The DNA sequence flanking this binding site. The putative E-box (CACGTG) was shown in pink.

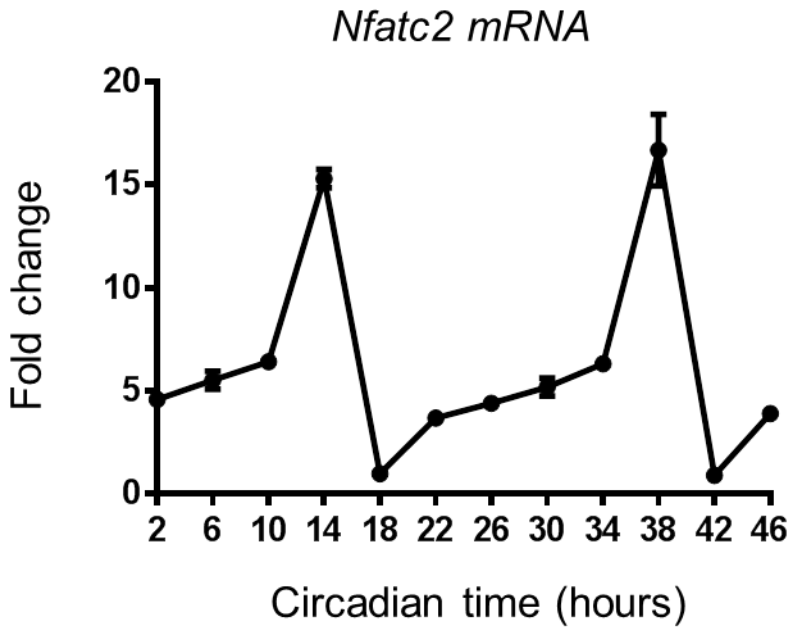


Figure S14, Plots of qRT-PCR data for *Nfatc2* in WT mouse hip cartilages collected at 4 hours intervals across two circadian cycles. Data shown as Mean ± SEM. N=2.

Supplementary Table 1, Lists of differentially expressed genes between WT and *Col2a1-Bmal1*$^{-/-}$ mice at the four time points studied (please see a separate supplementary excel file).

Supplementary Table 2, List of Taqman primers and antibodies.

Gene Name	Assay ID
Actb	Mm00607939_s1
Bmal1	Mm00500226_m1
Ctgf	Mm01192932_g1
Serpine1	Mm00435860_m1
Id3	Mm01188138_g1
Nfatc2	Mm00477776_m1
Sox9	Mm00448840_m1
Acan	Mm00545794_m1
Col2a1	Mm01309565_m1
Per2	Mm00478113_m1
Rev-erba	Mm00520708_m1

Protein Name	Manufacturer	Cat No
BMAL1 (IHC and IF)	Made in house– Sladek et al[47]	
BMAL1 (ChIP)	Cell Signaling Technology	#14020
CLOCK	Abcam	#Ab3715
pSMAD2	Cell Signaling Technology	#3108
pSMAD1/5	Cell Signaling Technology	#9516
NFATC2	Santa Cruz Biotechnology	sc-13034
SOX9	Millipore	AB5535