Supporting Information

Greenblatt et al. 10.1073/pnas.1320036110

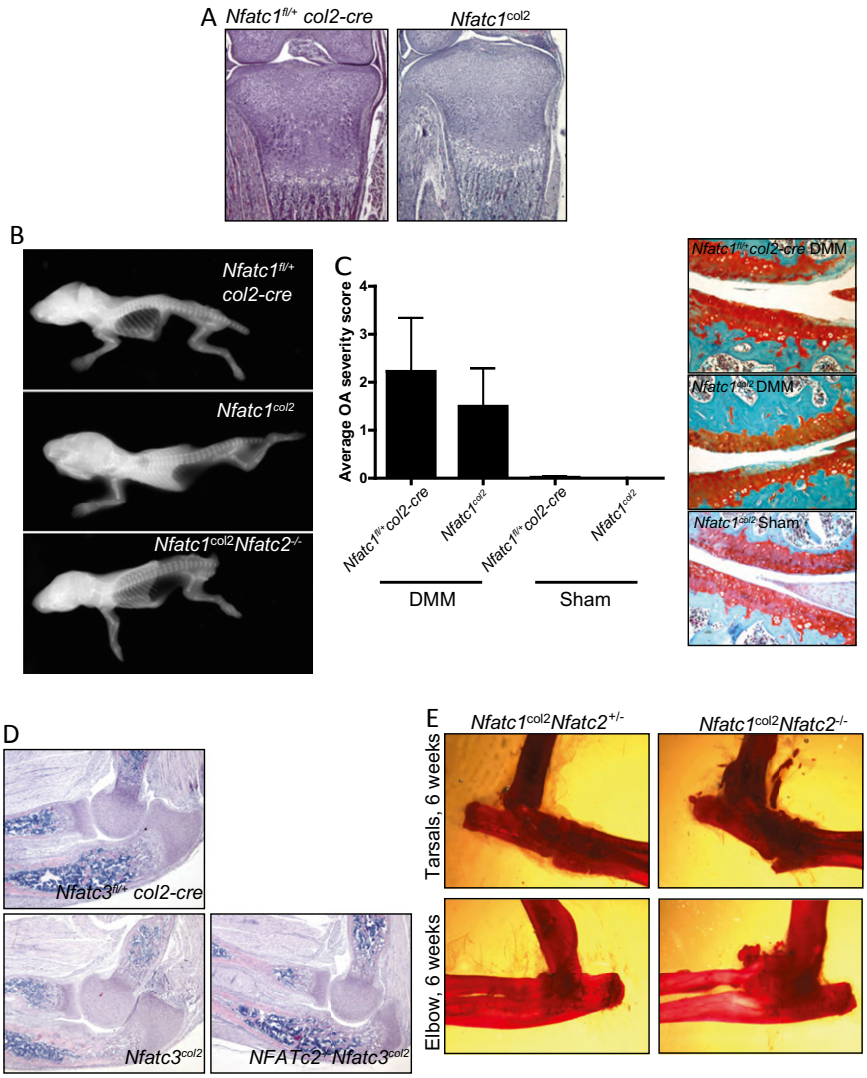

Fig. S1. Supplemental analysis of various nuclear factor of activated T cells (NFAT)-deficient mice including the destabilization of the medial meniscus (DMM) model and skeletal preparations of *Nfatc1*^{col2}*Nfatc2*$^{-/-}$ mice. (*A*) Micrographs of the proximal tibia of 1-wk-old mice of the indicated genotypes. (*B*) Radiographs of 3-d-old mice of the indicated genotypes. (*C Left*) Semiquantitative histologic scoring of osteoarthritis (OA) on the knee joints of mice using the Glasson system on mice age 18–20 wk of the indicated genotype that underwent DMM or sham surgery 8 wk earlier. (*Right*) Representative safranin O-stained histologic sections of the medial compartment of the knee of mice of the indicated genotype. (*D*) Micrographs from the elbow of 1-wk-old mice of the indicated genotypes. (*E*) Alizarin red-stained skeletal preparations of 6-wk-old mice of the indicated genotypes.

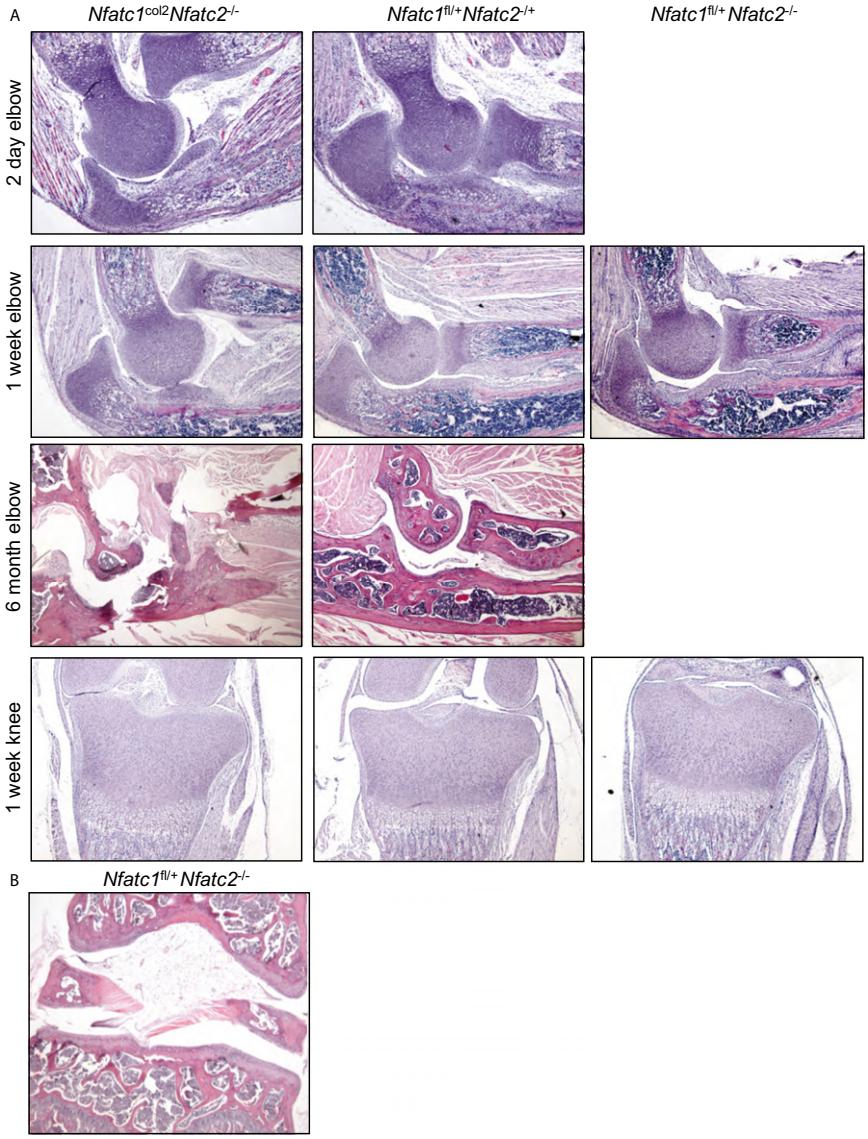

Fig. S2. Histologic characterization of *Nfatc1*^{col2}*Nfatc2*$^{-/-}$ mice. (*A*) Micrographs of H&E stains of the indicated anatomic sites from *Nfatc1*^{col2}*Nfatc2*$^{-/-}$ and littermate controls. (*B*) Micrographs of H&E stains of the knee of a *Nfatc1*$^{fl/+}$*Nfatc2*$^{-/-}$ mouse of the same cohort as that depicted in Fig. 2*E*.

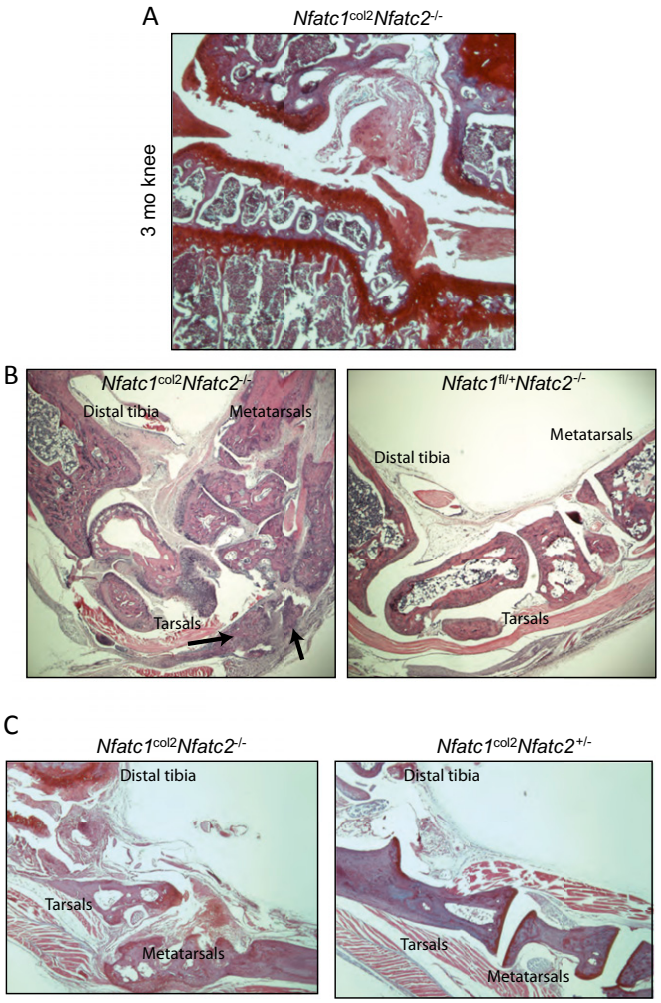

Fig. S3. Proteoglycan depletion in the knee and tarsals of *Nfatc1*^{col2}*Nfatc2*$^{-/-}$ mice. (*A*) Micrograph of a safranin O stain of the knee joint of a 3-mo-old *Nfatc1*^{col2}*Nfatc2*$^{-/-}$ mouse demonstrating cartilage loss. (*B*) Micrograph of H&E-stained sections of the ankle of 3-mo-old mice of the indicated genotypes. (*C*) Micrograph of a safranin O stain of the ankle of 3-mo-old mice of the indicated genotypes, demonstrating proteoglycan depletion from the articular surfaces and formation of ectopic cartilage around the sites of subluxation.

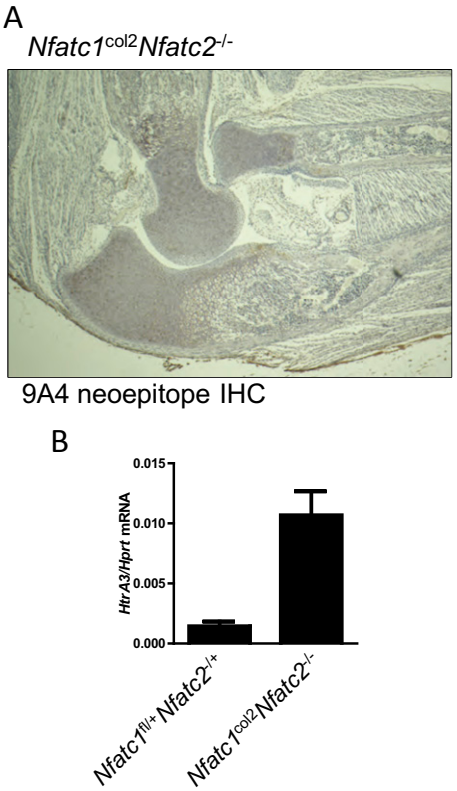

Fig. S4. Further molecular characterization of *Nfatc1*^{col2}*Nfatc2*$^{-/-}$ mice. (*A*) Micrograph of the elbow of 1-wk-old *Nfatc1*$^{fl/+}$*Nfatc2*$^{-/-}$ mice stained with the 9A4 antibody recognizing a collagenase-generated collagen neoepitope. This micrograph serves as an additional comparison with those in Fig. 3*B*. (*B*) Quantitative RT-PCR analysis for *Htra3* expression in elbow articular cartilage mRNA from 21-d-old mice. $n = 4$ mice per genotype. Values are mean + SD. By an unpaired t test, $P < 0.0001$.

Table S1. PCR primers used

Gene	Forward primer	Reverse primer
Col2a1	CGAGTGGAAGAGCGGAGAACT	AACTTTCATGGCGTCCAAGGT
Mmp13	CTTCTTCTTGTTGAGCTGGACTC	CTGTGGAGGTCACTGTAGACT
Adamts5	CGAAGAGCACTACGATGCAG	TTCATGAGCCACAGTGAAGG
ColXa1	TTCTGCTGCTAATGTTCTTGACC	GGGATGAAGTATTGTGTCTTGGG
Col9a1	ACCCTGGGTATCCGCAACT	ACCCTGGTAAGTCATCTTGGC
Sox9	AGGAAGCTGGCAGACCAGTA	TGTAATCGGGGTGGTCTTTC
HtrA1	CGTACCACGCTCCTGTCTTT	GGCACAGGTTGGTGTAGGTC
HtrA3	GGCACAGACGGGCATACTTA	ACTGGTCAGCTGGTGGAGAC
Prg4	GGGTGGAAAATACTTCCCGTC	CAGGACAGCACTCCATGTAGT
Human *NFAT5*	GGGTCAAACGACGAGATTGTG	TTGTCCGTGGTAAGCTGAGAA
Human *NFATc2*	CAGAATGCCACGAGCCAAAGA	GGTCCTGAAAACTCCTTCCTGA
Human *NFATc1*	CAGCGGAGGAAGAACACTATG	GTTATCTCGATGCGAGGACT