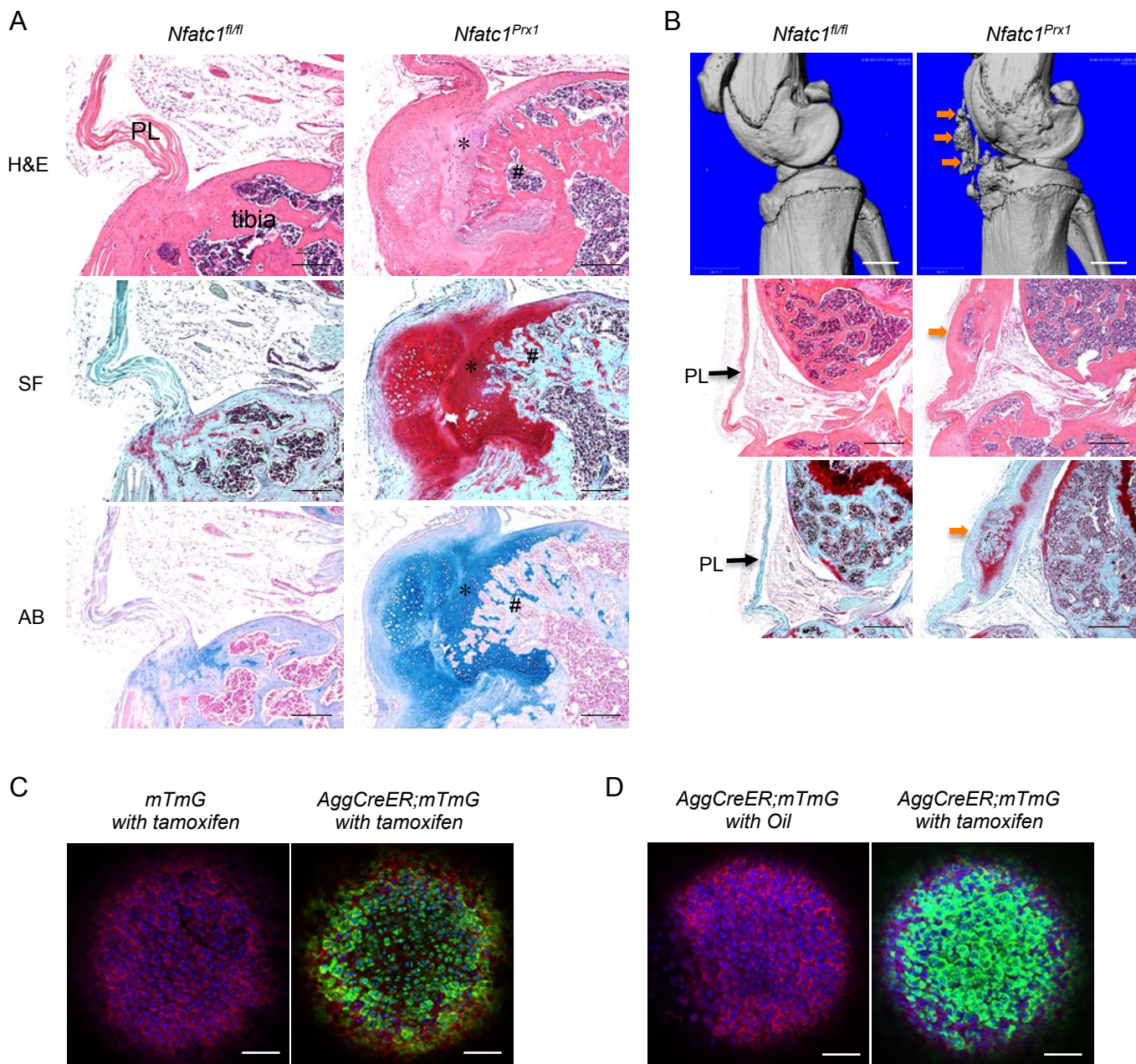
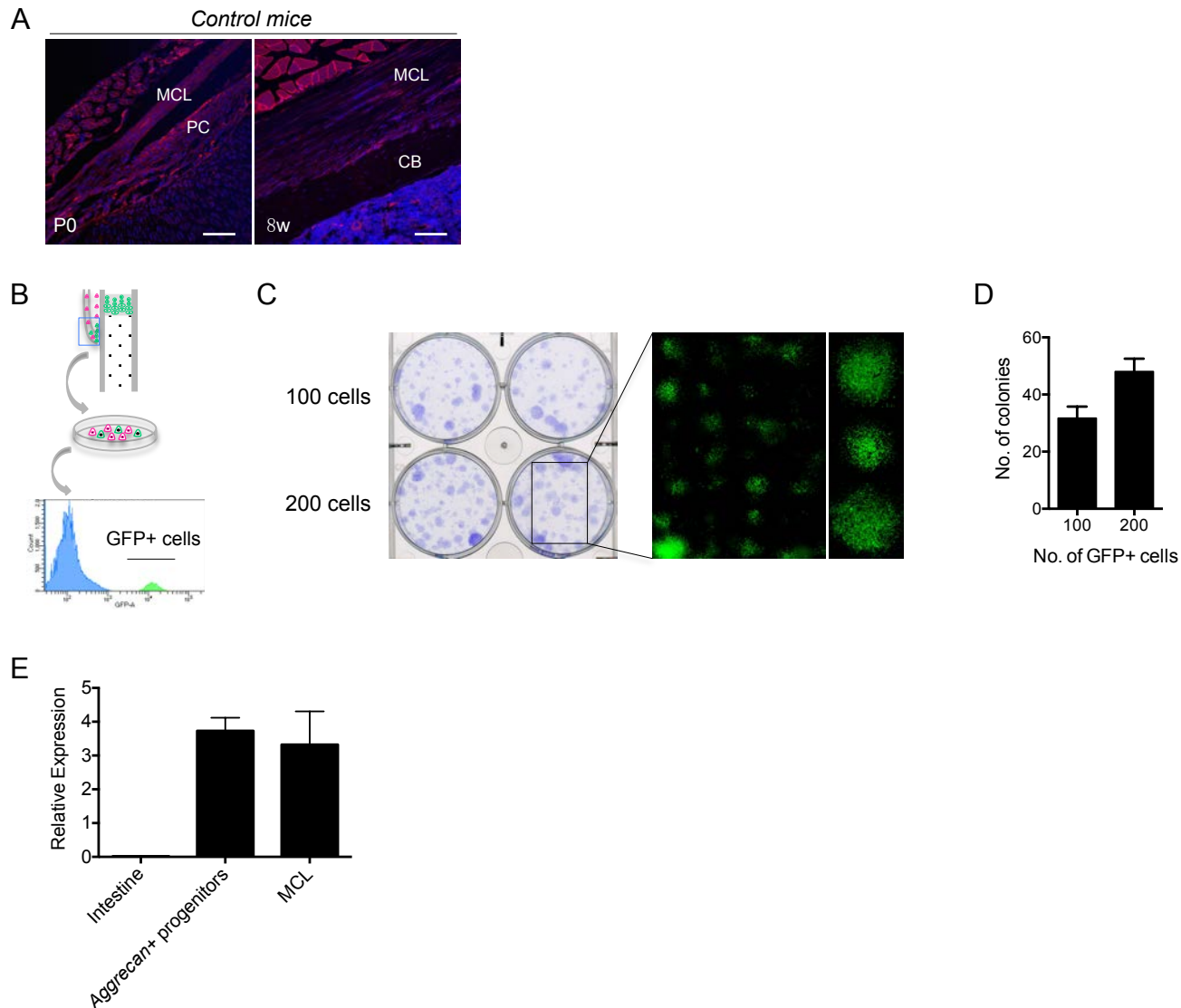


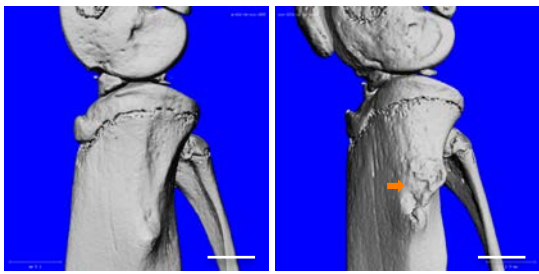


**Supplemental Figure 1. Osteochondroma formation after deleting nuclear factor of activated T cells c1 (*Nfatc1*) in limb mesenchymal progenitors and postnatal *Aggrecan*-expressing cells. (A) H&E, safranin O/fast green (SF) and alcian blue (AB) staining of sagittal sections of knee joints from 16-week-old *Nfatc1<sup>fl/fl</sup>* and *Nfatc1<sup>Prx1</sup>* mice showing osteochondroma formation at the tibial enthesis of patellar ligament (PL) in *Nfatc1<sup>Prx1</sup>* mice (stars, cartilage cap; pounds, marrow cavity). Scale bars: 100  $\mu$ m. (B)  $\mu$ CT images and H&E, SF stain showing extraskeletal osteochondroma formation within PL (orange arrows) around knee joints of *Nfatc1<sup>Prx1</sup>* mice. Scale bars: 1 mm (upper panels), 200  $\mu$ m (middle and lower panels). Images are representative of 8 mice per genotype. (C and D) Confocal images of articular cartilage of hip heads from *AggCreER;mTmG* mice at 2 weeks (C) and 4 weeks (D) after tamoxifen administration. Control animals (*mTmG* mice with tamoxifen; *AggCreER;mTmG* mice with sunflower oil) did not show any GFP labeling. Scale bars: 5 mm. Images are representative of 3 mice per genotype.**

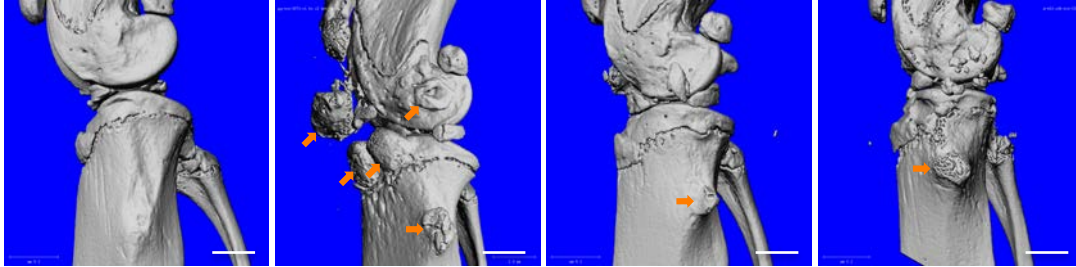


**Supplemental Figure 2. Identifying AggreCAN- and nuclear factor of activated T cells c1 (NFATc1)-expressing cells at the enthesis as precursors of osteochondromas.** (A) Fluorescent images of control (*mTmG*) mice. (B) Schematic showing the isolation of *AggreCAN*-expressing cells from the tibial medial collateral ligament (MCL) enthesis of *AggCreER;mTmG* mice 1 week after tamoxifen. GFP+ cells were flow-sorted after 7 days of ex vivo expansion. (C and D) Colony-forming units of *AggreCAN*<sup>+</sup> cells. n = 3 animals. Scale bars, 100  $\mu$ m. (E) Real-time PCR determining the expression of *Scleraxis* gene in *AggreCAN*-expressing enthesal progenitors. The mouse intestine tissue was used as a negative control, and tissue from the MCL and its enthesis as the positive control.

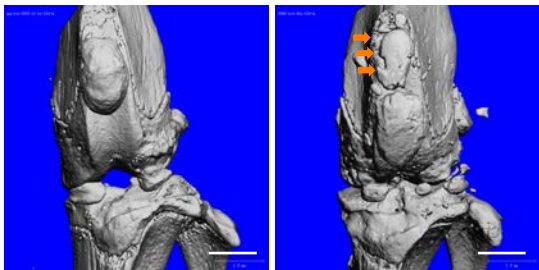
A

*Nfatc1<sup>fl/fl</sup>**Nfatc1<sup>Scx</sup>*

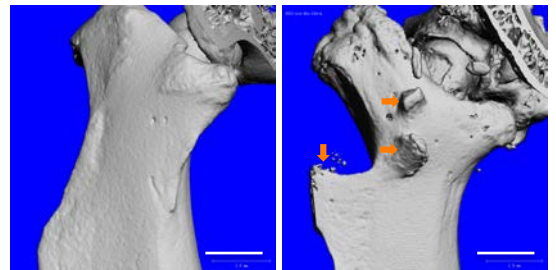
B

*Nfatc1<sup>fl/fl</sup>Nfatc2<sup>+/-</sup>**Nfatc1<sup>Scx</sup>Nfatc2<sup>+/-</sup>**Nfatc1<sup>fl/fl</sup>Nfatc2<sup>-/-</sup>**Nfatc1<sup>Scx</sup>Nfatc2<sup>-/-</sup>*

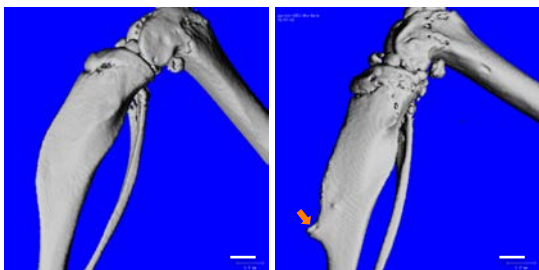
C

*Nfatc1<sup>fl/fl</sup>Nfatc2<sup>-/-</sup>**Nfatc1<sup>Scx</sup>Nfatc2<sup>-/-</sup>*

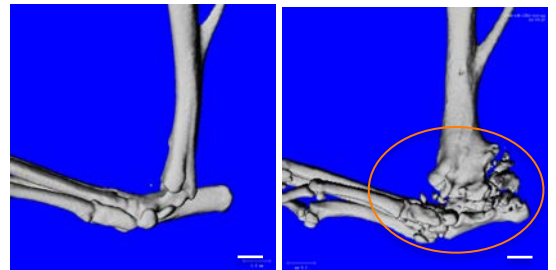
D

*Nfatc1<sup>fl/fl</sup>Nfatc2<sup>-/-</sup>**Nfatc1<sup>Scx</sup>Nfatc2<sup>-/-</sup>*

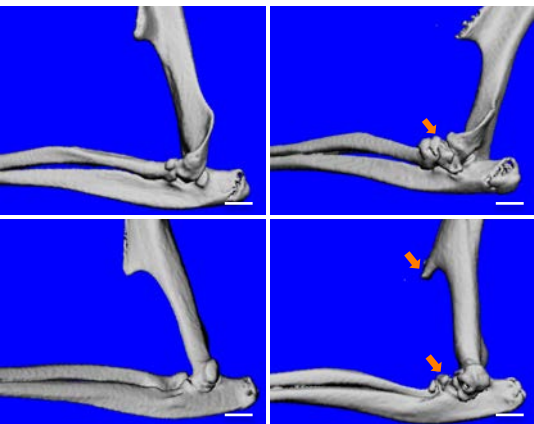
E

*Nfatc1<sup>fl/fl</sup>Nfatc2<sup>-/-</sup>**Nfatc1<sup>Scx</sup>Nfatc2<sup>-/-</sup>*

F

*Nfatc1<sup>fl/fl</sup>Nfatc2<sup>-/-</sup>**Nfatc1<sup>Scx</sup>Nfatc2<sup>-/-</sup>*

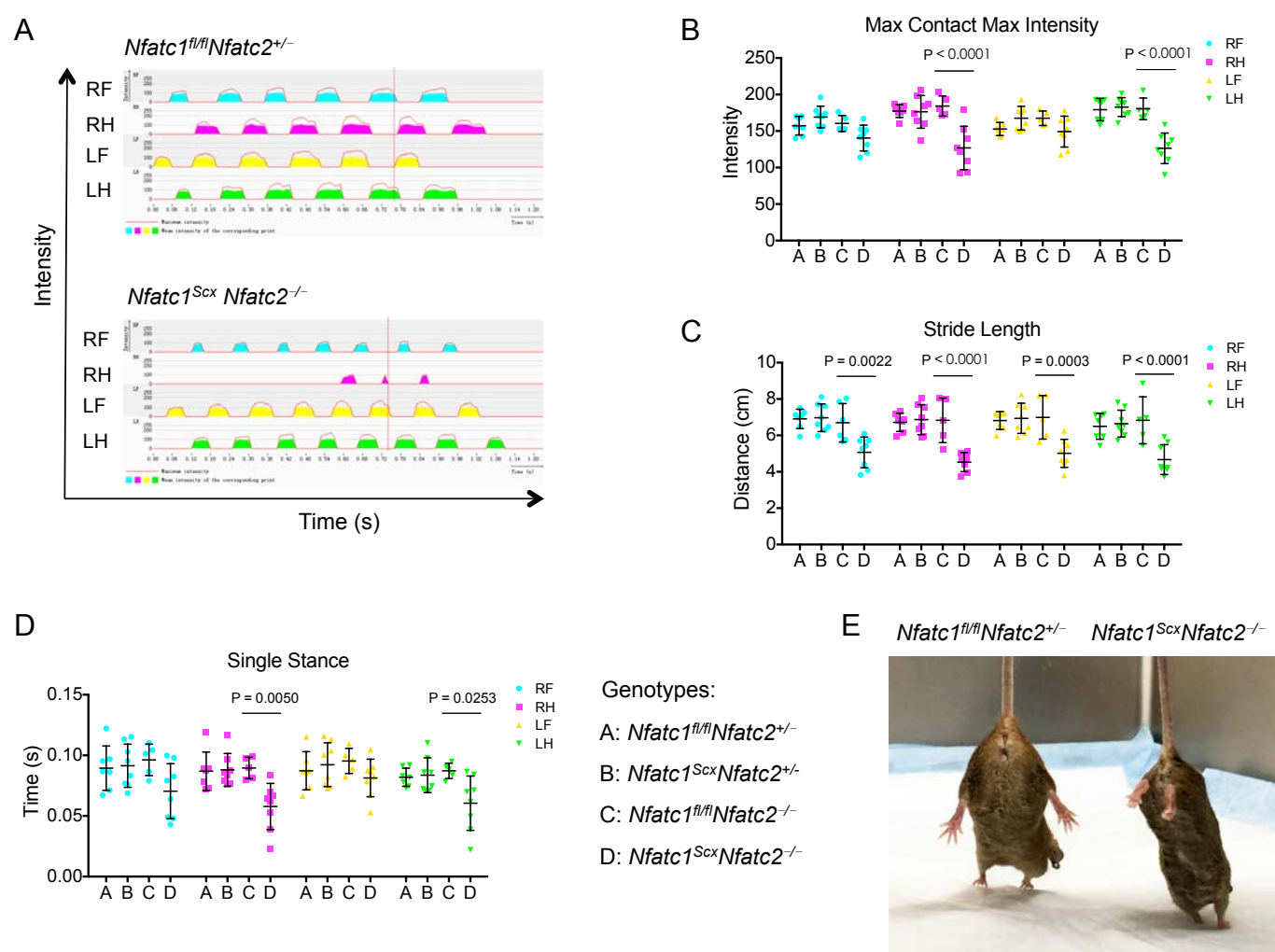
G

*Nfatc1<sup>fl/fl</sup>Nfatc2<sup>-/-</sup>**Nfatc1<sup>Scx</sup>Nfatc2<sup>-/-</sup>*

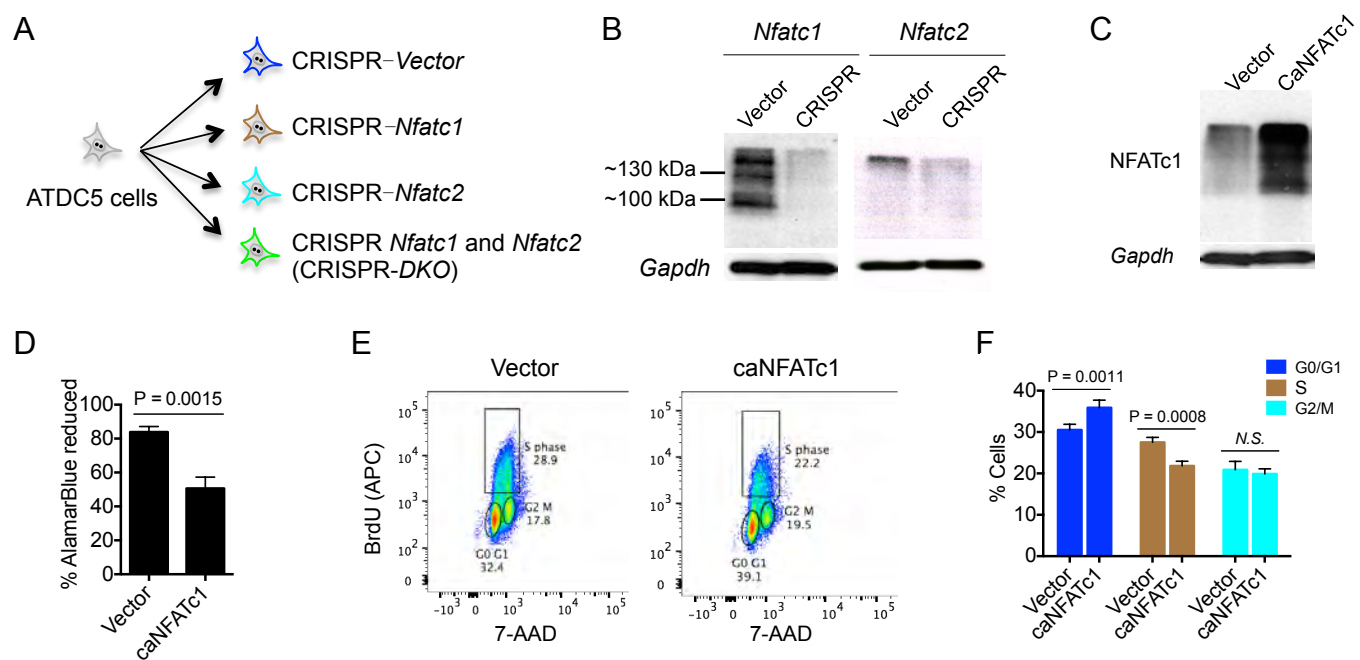
H

*Nfatc1<sup>fl/fl</sup>Nfatc2<sup>-/-</sup>**Nfatc1<sup>Scx</sup>Nfatc2<sup>-/-</sup>*

**Supplemental Figure 3. Osteochondroma formation after deleting nuclear factor of activated T cells c1 (NFATc1) in Scx-expressing enthesal progenitors on a wild-type or *Nfatc2*-deficient background.** (A) Representative  $\mu$ CT images showing osteochondroma formation at the tibial medial collateral ligament entheses in 16-week-old *Nfatc1<sup>Scx</sup>* mice. (B) Representative  $\mu$ CT images of osteochondroma formation at proximal tibia and epiphyses of knee joints (arrows). (C-E) Representative  $\mu$ CT images of osteochondroma formation at distal femur (C), proximal femur (D) and distal tibia (E) (arrows). (F) Representative  $\mu$ CT images of subluxation/dislocation of ankle joints in 16-week-old *Nfatc1<sup>Scx</sup>Nfatc2<sup>-/-</sup>* mice. (G) Representative  $\mu$ CT images of osteochondroma formation at elbow joint and proximal humerus. (H) Representative images of 16-week-old *animals* displaying deformation of the ankle joint in *Nfatc1<sup>Scx</sup>Nfatc2<sup>-/-</sup>* mice compared to *Nfatc1<sup>fl/fl</sup>Nfatc2<sup>-/-</sup>* mice. Scale bars: 1.0 mm. Images are representative of 8 (*Nfatc1<sup>fl/fl</sup>Nfatc2<sup>+/-</sup>*), 8 (*Nfatc1<sup>Scx</sup>Nfatc2<sup>+/-</sup>*), 9 (*Nfatc1<sup>fl/fl</sup>Nfatc2<sup>-/-</sup>*), 10 (*Nfatc1<sup>Scx</sup>Nfatc2<sup>-/-</sup>*) animals.



**Supplemental Figure 4. Musculoskeletal dysfunction after deleting nuclear factor of activated T cells c1 and c2 (NFATc1 and NFATc2) in Scleraxis-expressing entheselial progenitors. (A-D)** Gait analyses of 8-week-old mice measuring contact intensity (A and B), stride length (C) and time of single stance (D) in mice of the indicated genotypes. n = 7 (A), 8 (B), 5 (C), 8 (D) animals. P values determined by two-way ANOVA followed by Tukey's tests for multiple comparisons. (E) Representative image of decreased hindlimb abduction in 12-week-old *Nfatc1<sup>Scx</sup>Nfatc2<sup>-/-</sup>* mice.



**Supplemental Figure 5. Nuclear factor of activated T cells c1 (NFATc1) inhibits ATDC5 cell proliferation by blocking G1/S phase progression.** (A and B) Deletion of *Nfatc1* and/or *Nfatc2* in ATDC5 cells by CRISPR/Cas9 lentiviruses, confirmed by Western blotting. (C) Western blotting of ATDC5 cells overexpressing constitutively active NFATc1 (caNFATc1) by retroviral transduction. (D-F) Cell proliferation (D) and cell cycle (E and F) of ATDC5 cells overexpressing caNFATc1. n = 3 per sample. Two-tailed Student's t tests were performed for D. P values in F were determined by Two-Way ANOVA followed by Sidak's test.

**Supplemental Table 1.** Summary of bilateral osteochondromas at the tibial medial collateral ligament (MCL) enthesis from mice with combinatorial deletion of *Nfatc1* and *Nfatc2* in postnatal *Aggrecan*-expressing cells. Tamoxifen was given at 8- or 12-weeks of age.

| Osteochondromas at tibial MCL entheses<br>3 months after tamoxifen |            |           |                |
|--------------------------------------------------------------------|------------|-----------|----------------|
| Genotypes                                                          | Unilateral | Bilateral | % of bilateral |
| <i>Nfatc1<sup>fl/fl</sup>Nfatc2<sup>+/-</sup></i>                  | 3/10       | 0/10      | 0%             |
| <i>Nfatc1<sup>fl/fl</sup>Nfatc2<sup>-/-</sup></i>                  | 5/11       | 2/11      | 18%            |
| <i>Nfatc1<sup>AggCreER</sup>Nfatc2<sup>+/-</sup></i>               | 4/10       | 5/10      | 50%            |
| <i>Nfatc1<sup>AggCreER</sup>Nfatc2<sup>-/-</sup></i>               | 3/13       | 10/13     | 77%            |

**Supplemental Table 2. Real-time PCR primers used**

| Gene                   | Forward primer (5'-3')    | Reverse primer (5'-3')     |
|------------------------|---------------------------|----------------------------|
| <i>Hprt</i>            | GTTAAGCAGTACAGCCCCAAA     | AGGGCATATCCAACAACAAACTT    |
| <i>Fabp4</i>           | AAGGTGAAGAGCATCATAACCCT   | TCACGCCTTTCATAACACATTCC    |
| <i>Lpl</i>             | GGGAGTTTGGCTCCAGAGTTT     | TGTGTCTTCAGGGGTCCTTAG      |
| <i>Cd36</i>            | ATGGGCTGTGATCGGAACTG      | GTCTTCCCAATAAGCATGTCTCC    |
| <i>Ibsp</i>            | AAAGTGAAGGAAAGCGACGA      | GTTCTTCTGCACCTGCTTC        |
| <i>Sp7</i>             | ATGGCGTCCTCTCTGCTTGA      | GAAGGGTGGGTAGTCATTTG       |
| <i>Spp1</i>            | AGCAAGAACTCTTCCAAGCAA     | GTGAGATTCGTCAGATTCATCCG    |
| <i>Col2a1</i>          | CGAGTGGAAGAGCGGAGAACT     | AACTTTCATGGCGTCCAAGGT      |
| <i>Acan</i>            | CTACAGAACAGCGCCATCAT      | CTACAGAACAGCGCCATCAT       |
| <i>Scx<sup>A</sup></i> | CCTTCTGCCTCAGCAACCAG      | GGTCCAAAGTGGGGCTCTCCGTGACT |
| <i>Cdkn1a</i>          | TCAGAGTCTAGGGGAATTGGA     | AATCACGGCGCAACTGCT         |
| <i>CyclinD1</i>        | GCGTACCCTGACACCAATCTC     | CTCCTCTTCGCACTTCTGCTC      |
| <i>CyclinD2</i>        | GAGTGGGAACTGGTAGTGTTG     | CGCACAGAGCGATGAAGGT        |
| <i>Cdk4</i>            | TGGCTGCCACTCGATATGAAC     | CCTCAGGTCCTGGTCTATATG      |
| <i>Cdk6</i>            | GGCGTACCCACAGAAACCATA     | AGGTAAGGGCCATCTGAAAAC      |
| <i>c-Myc</i>           | TGAGGAGACACCGCCAC         | CAACATCGATTTCTTCCTCATCTTC  |
| <i>Col10a1</i>         | TTCTGCTGCTAATGTTCTTGACC   | GGGATGAAGTATTGTGTCTTGGG    |
| <i>Mmp13</i>           | CTTCTTCTTGTTGAGCTGGACTC   | CTGTGGAGGTCAGTGTAGACT      |
| <i>Tnsp1</i>           | CACAATATCAAGGATATCGACGTGA | ACATCAGTTCTGTTCTTCGGGTACA  |
| <i>Col1a1</i>          | ACTGTCCCAACCCCCAAAG       | ACGTATTCTTCCGGGCAGAA       |
| <i>Comp</i>            | TGTGGAAACAGATGGAGCAG      | CCGGGACCTGTAGAGGACTT       |
| <i>Bglap</i>           | GGGCAATAAGGTAGTGAACAG     | GCAGCACAGGTCCTAAATAGT      |

<sup>A</sup> Guerquin MJ, et al. *J Clin Invest.* 2013(8);123:3564-3576.