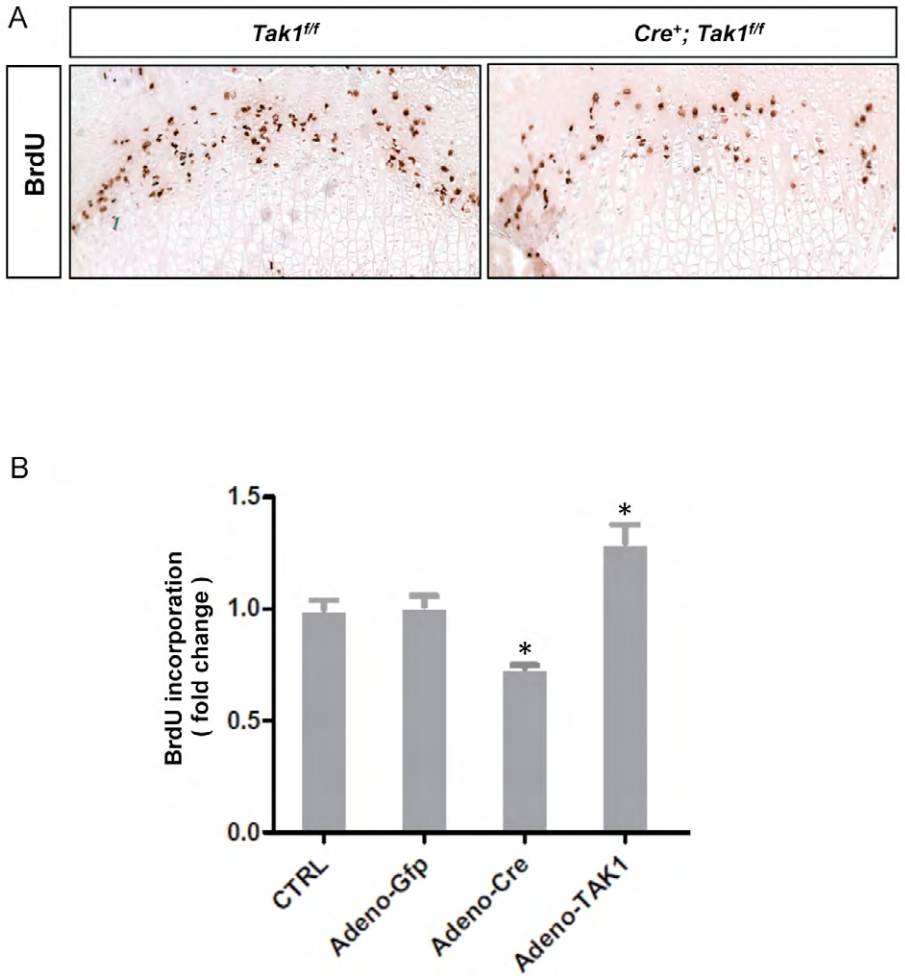

Fig. S1. Chondrocyte-specific deletion of *Tak1* results in reduced chondrocyte proliferation. (A) BrdU immunohistochemistry of growth plate cartilage sections from one-month-old *Col2a1-CreER^{T2}; Tak1$^{f/f}$* (*Cre^{+}; Tak1$^{f/f}$*) mice and Cre-negative control littermates (*Tak1$^{f/f}$*) injected with Tamoxifen at one week of age and with BrdU three hours prior to sacrifice. **(B)** Primary sternal chondrocytes isolated from *Tak1$^{f/f}$* mice were infected with adenovirus encoding GFP (Ad-GFP), Cre recombinase (Ad-Cre), or TAK1 (Ad-TAK1). After 72 hours, cells were labeled with BrdU for 4 hours and subsequently harvested for BrdU incorporation analysis. **P*<0.01, one-way ANOVA followed by Newman-Keuls post test.

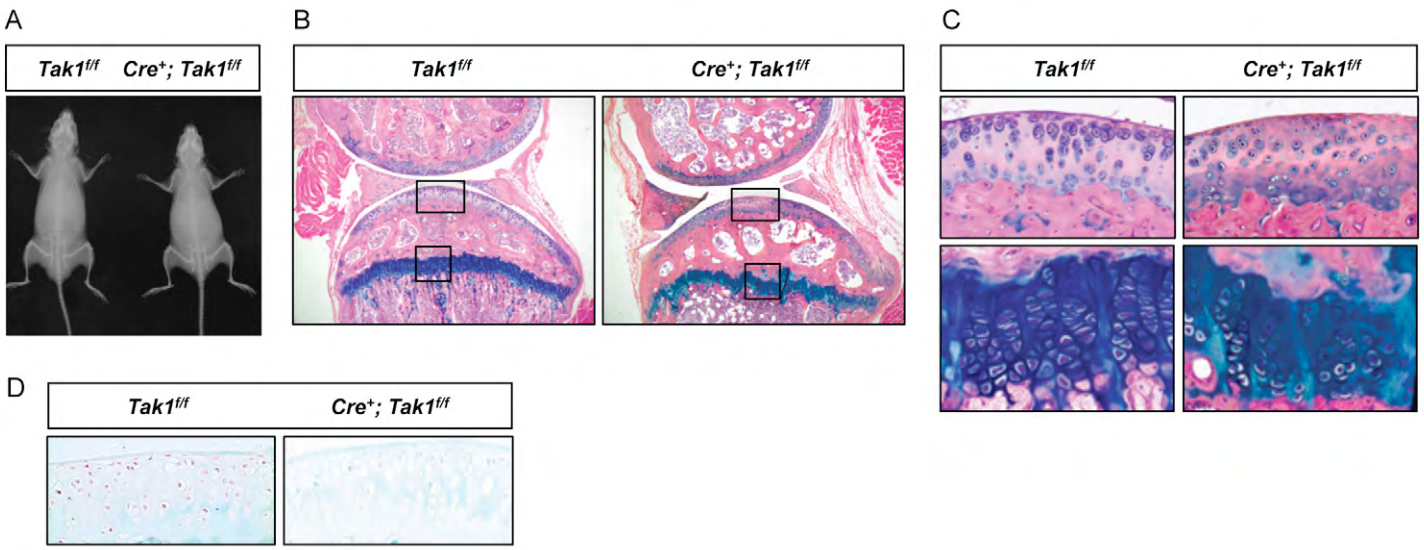

Fig. S2. Postnatal chondrocyte-specific deletion of *Tak1* results in reduced proteoglycan content in the articular cartilage at three months of age. (A) Radiographic analyses of three-month-old *Col2a1-CreER^{T2}; Tak1$^{f/f}$* (*Cre^{+}; Tak1$^{f/f}$*) mice and Cre-negative control littermates (*Tak1$^{f/f}$*) following injection with Tamoxifen at one week of age. **(B)** 5× images of Alcian blue/hematoxylin/Orange G staining of knee joint sections from three-month-old *Col2a1-CreER^{T2}; Tak1$^{f/f}$* (*Cre^{+}; Tak1$^{f/f}$*) mice and Cre-negative control littermates (*Tak1$^{f/f}$*) injected with Tamoxifen at one week of age. **(C)** High magnification images (20×) of the articular cartilage (upper panels) or growth plate cartilage (lower panels) from corresponding boxed regions in panel B. **(D)** SOX9 immunohistochemistry of articular cartilage sections from three-month-old *Col2a1-CreER^{T2}; Tak1$^{f/f}$* (*Cre^{+}; Tak1$^{f/f}$*) mice and Cre-negative control littermates (*Tak1$^{f/f}$*) injected with Tamoxifen at one week of age.

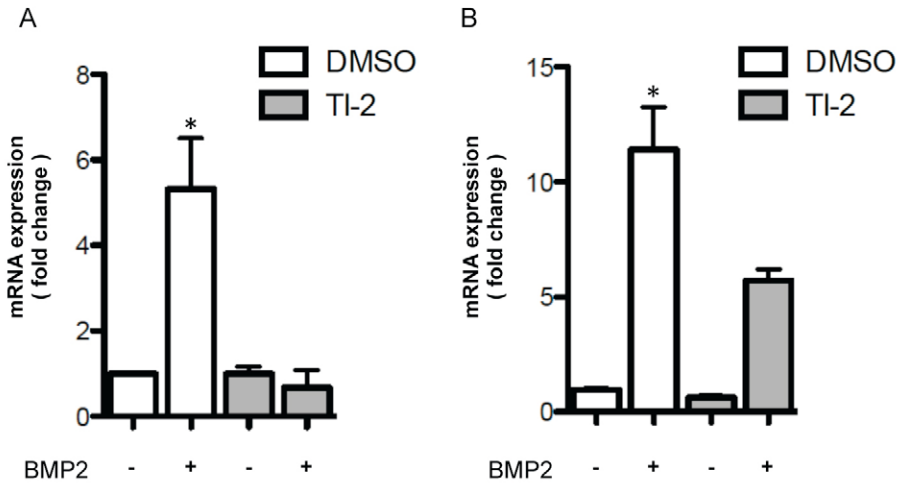

Fig. S3. TAK1 kinase activity positively regulates BMP2-mediated *Sox9* gene expression. RCS cells were starved of serum for 12 hours and then treated with vehicle, 5Z-7-oxozeanol (TI-2, 3 μM) alone, BMP2 (100 ng/ml) alone, or TI-2 and BMP2 in combination for either 3 hours **(A)** or 24 hours **(B)**. Total RNA was harvested from the cultures for quantitative real-time RT-PCR analysis of *Sox9* gene expression. **P*<0.01, one-way ANOVA followed by Newman-Keuls post test.

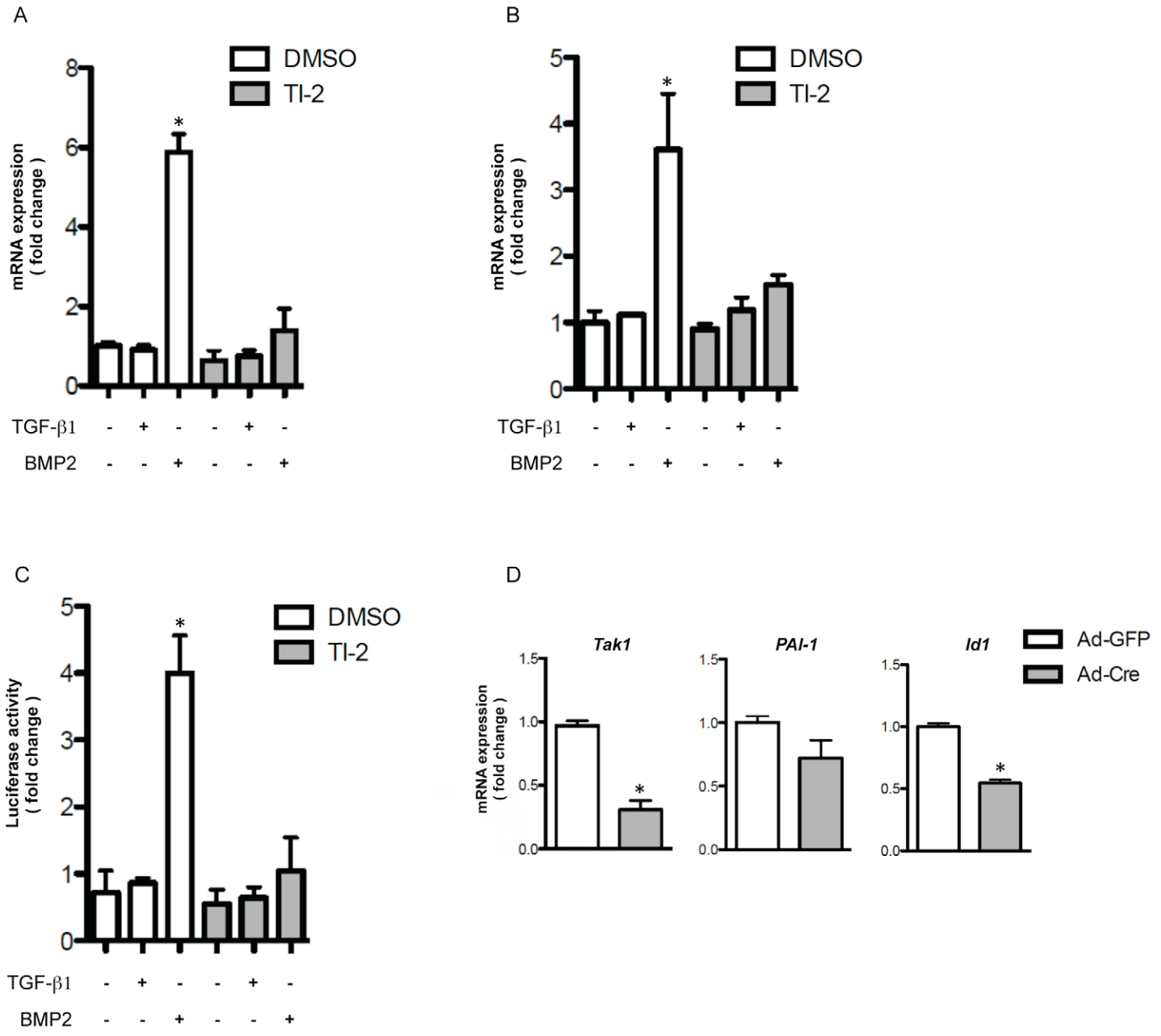

Fig. S4. TGF-β1 does not induce *Sox9* gene expression in committed chondrocytes. RCS cells **(A)** or wild type primary sternal chondrocytes **(B)** were starved of serum for 12 hours and then treated with vehicle, 5Z-7-oxozeanol (TI-2, 3 μM) alone, BMP2 (100 ng/ml) alone, TGF-β1 (5 ng/ml) alone, TI-2 and BMP2 in combination, or TI-2 and TGF-β1 in combination for 3 hours. Total RNA was harvested from the cultures for quantitative real-time RT-PCR analysis of *Sox9* gene expression. **P*<0.01, one-way ANOVA followed by Newman-Keuls post test. **(C)** Luciferase reporter assay using lysates from RCS cells transfected with a luciferase reporter construct containing a 1.0 kb fragment of the *Sox9* promoter. The cells were serum starved for 12 hours and then treated with either vehicle, 5Z-7-oxozeanol (TI-2, 3 μM) alone, BMP2 (100 ng/ml) alone, TGF-β1 (5 ng/ml) alone, TI-2 and BMP2 in combination, or TI-2 and TGF-β1 in combination for 8 hours. **P*<0.05, one-way ANOVA followed by Newman-Keuls test. **(D)** Primary sternal chondrocytes from *Tak1*$^{f/f}$ mice were infected with adenovirus encoding GFP (Ad-GFP) or Cre-recombinase (Ad-Cre). Forty-eight hours later, cells were harvested for quantitative real-time RT-PCR analyses of the indicated genes. **P*<0.05, Student's t-test.

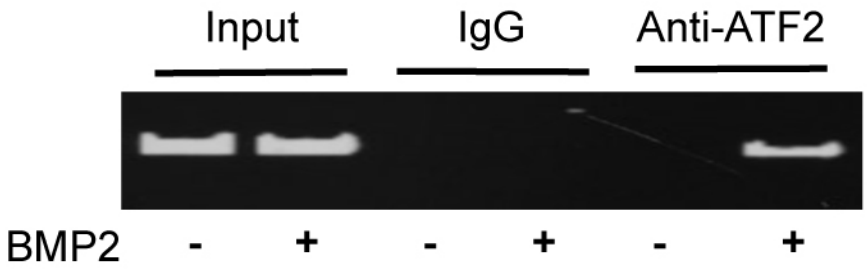

Fig. S5. ATF2 binds to the *Sox9* promoter in response to BMP2 signaling. ChIP assay using cell lysates from primary sternal chondrocytes serum starved for 12 hours and then treated with vehicle or BMP2 (100 ng/ml) for 90 minutes. PCR amplification of chromatin from these lysates before (input) or after immunoprecipitation with an anti-ATF2 antibody or non-immune IgG is shown. PCR primers are described in Fig. 6C.

Table S1. Primers used for RT-PCR

Mouse primers

Tak1	5'-CTGCCAGTGAGATGATCG-3' 5'-CAGGCTCCATACAACTTGAC-3'
Aggrecan I	5'-CCTGCTACTTCATCGACCCC-3' 5'-AGATGCTGTTGACTCGAACCT-3'
Col2a1	5'-CCACACCAAATTCCTGTTCA-3' 5'-ACTGGTAAGTGGGGCAAGAC-3'
Col10a1	5'-CTTTGTGTGCCTTTCAATCG-3' 5'-GTGAGGTACAGCCTACCAGTTTT-3'
Col1a1	5'-TGGTTTGGAGAGAGCATGACCGA-3' 5'-TTGGTCGATGTAGGCTACGCTGTT-3'
Col9a1	5'-CGACCGACCAGCACATCAA-3' 5'-AGGGGGACCCTTAATGCCT-3'
Adamts5	5'-CCCAGGATAAAACCAGGCAG-3' 5'-CGGCCAAGGGTTGTAAATGG-3'
Mmp13	5'-TTTGAGGACACGGGGAAGA-3' 5'-ACTTTGTCGCCAATTCCAGG-3'
Sox9	5'-AGGAAGCTGGCAGACCAGTA-3' 5'-CGTTCTTCACCGACTTCCTC-3'
Sox5	5'-ATGGAAGTCGATGGCAATAAAGT-3' 5'-CCACCACATCCGCTAAGCTG-3'
Sox6	5'-AATGCACAACAAACCTCACTCT-3' 5'-AGGTAGACGTATTTCGGAAGGA-3'
Id1	5'-TGGACGAACAGCAGGTGAACG-3' 5'-GCACTGATCTCGCCGTTCAGG-3'
Pai-1	5'- GGTCATGGAACAAGAATG -3' 5'- GCTGAGACTAGAATGGCTG -3'
β-actin	5'-AGATGTGGATCAGCAAGCAG-3' 5'-GGGCAAGTTAGGTTTTGTCA-3'
Atf2	5'-CTACGAGGGGCGTCAGAGTA-3'

	5’-GGGGAATCAATGAAAACCAA-3’

Rat primers

Sox9	5’-CGCCATCTTCAAGGCGCT-3’
	5’-GTGTAGTCGTACTGTGAG-3’
β-actin	5’-GCTACAGCTTCACCACCACA-3’
	5’-ATCGTACTCCTGCTTGCTGA-3’