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Elimination of BMP7 from the developing limb mesenchyme leads to articular cartilage degeneration and synovial inflammation with increased age

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ABSTRACT

While osteo- and chondro-inductive activities of recombinant human bone morphogenetic protein 7 are well established, evaluation of the role of endogenous BMP7 in skeletal homeostasis has been hampered by perinatal lethality in BMP7 knockout mice. Here, we examined physiological roles of endogenous BMP7 in joint homeostasis and showed that proteoglycan contents in articular cartilage were significantly reduced in the absence of BMP7. Loss of BMP7 did not affect survival of articular cartilage cells, but resulted in reduced expression of aggrecan and enhanced expression of matrix metalloproteinase 13. We also found extensive synovial hyperplasia and enhanced expression of Activin A. These findings suggest that locally produced BMP7 is prerequisite for postnatal synovial joint homeostasis and may be involved in osteoarthritic changes in adults.
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1. Introduction

Osteoarthritis (OA) is a degenerative joint disease that severely decreases the activity of daily life (ADL). More than 25 million people suffer from OA, with prevalence in men and women over 40 years of age of 42.6% and 62.4%, respectively in Japan [1]. Although OA is multifactorial in initiation, articular cartilage degeneration is a major cause of OA, and maintenance of articular cartilage function is the most urgent and challenging need in this field.

Bone morphogenetic proteins (BMPs), multifunctional signaling molecules belonging to the transforming growth factor (TGF)-β gene superfamily, were identified by their ability to induce ectopic bone and cartilage in vivo [2–4]. We and other research groups have reported that osteogenic BMPs such as BMPs 2, 4, 5, 6, and 7 are expressed and play important roles during skeletogenesis and in postnatal bone homeostasis [5–14]. In addition, proper BMP signaling is required for postnatal joint homeostasis as Rountree et al. showed that articular cartilage gradually wears away in the absence of type Ia BMP receptor (Alk3) [15]. Among the BMP molecules, bone morphogenetic protein 7 (BMP7) is abundantly expressed in articular cartilage and synovial membrane obtained from patients undergoing autologous chondrocyte implantation [16]. Intra-articular injection of recombinant human BMP7 protein (rhBMP7) has been shown to promote survival of articular chondrocytes in an acute cartilage trauma model in sheep

Abbreviations: BMP7, bone morphogenetic protein 7; MMP13, matrix metalloproteinase 13; OA, osteoarthritis

Author's contribution: AK carried out the animal experiments, analyzed the results, and drafted the manuscript. TM, KM, JY, YM, MI, IS, DG, AE, and VR contributed experimental design and discussion. KT designed the initial plan for the study, conducted the experiments, participated in the evaluation of the results, and completed the final manuscript. All authors read and approved the final manuscript.

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[17]. And recent studies indicated that intra-articular administration of rhBMP7 significantly inhibited articular cartilage degeneration and osteophyte formation within the joint [18,19], and also blocked production of inflammatory cytokines by the synovial membrane [20] in vivo. These results strongly suggest that BMP7 may have a unique and pivotal role(s) in joint homeostasis through its anabolic/anti-catabolic, anti-apoptotic, and anti-inflammatory effects on specific joint tissues.

In spite of evidence that treatment with rhBMP7 positively affects joint homeostasis, the role(s) of endogenous locally produced BMP7 in this process have not been studied extensively. To define the role(s) of BMP7 in postnatal bone and joint homeostasis, we established limb mesenchyme-specific BMP7 conditional knockout (*Bmp7$^{c/c}$;Prx1::cre*) mice by breeding the mice carrying floxed Bmp7 alleles and cre recombinase driven by Prx1 enhancer and previously reported that endogenous BMP7 activity was dispensable in both skeletal development and postnatal bone homeostasis including the process of fracture healing [13]. Here we show that the absence of BMP7 led to a significant reduction in the amount of proteoglycan and aggrecan present in articular cartilage while the cartilage degrading enzyme, matrix metalloprotease 13 (MMP-13), is increased. We also observe extensive synovial hyperplasia and macrophage infiltration. Our data strongly suggest that loss of locally produced BMP7 leads to degenerative changes in articular cartilage that correlate with the development of age-related OA.

2. Materials and methods

2.1. Animals

All mouse experiments were approved and conducted in accordance with the guidelines of the animal committee of Tokyo Medical and Dental University.

Limb specific *Bmp7$^{c/c}$;Prx1::cre* mice were generated as described previously [13,21,22]. Briefly, mice carrying a floxed allele of BMP7 were bred to Prx1::cre mice to obtain *Bmp7$^{c/c}$;Prx1::cre* mice. Mouse genotypes were determined as described previously [13]. Mice were housed under a 12-h light–dark cycle and allowed food and water *ad libitum*. Littermates not expressing cre recombinase were designated as experimental controls.

2.2. Histology

Bmp7$^{c/c}$;Prx1::cre and control mice were sacrificed at 4, 8 and 24 weeks of age. The right hind limb of each experimental sample was fixed in 4% paraformaldehyde (PFA), de-calcified, and embedded in paraffin [23]. Five μm sagittal sections were prepared from the medial side of the knee joint, de-paraffinized in xylene, rehydrated through graded alcohols, and stained with safranin-o/fast green or hematoxylin/eosin to evaluate the morphological changes of knee joint. All sections in this study were visualized by using an Olympus BX 53 microscope (Olympus, Tokyo, Japan).

2.3. Semi quantitative evaluation of cartilage degeneration and synovial inflammation

The severity of articular cartilage degeneration was assessed according to modified Mankin's scoring system (Supplemental Table 1) [24]. The severity of cell infiltration and synovial hyperplasia was assessed according to a validated synovitis scoring system (Supplemental Table 2) [25].

2.4. Immunohistochemistry

Immunohistological analyses were performed as described previously [23]. Briefly, de-paraffinized and re-hydrated sections were pretreated with 0.4 mg/ml of proteinase K (DAKO, Carpinteria, CA, USA) in Tris–HCl buffer for 5 min at room temperature for antigen retrieval. All subsequent incubations were performed in a humidified chamber. Endogenous peroxidases were quenched using 0.3% hydrogen peroxidase in methanol for 30 min at room temperature. Any residual enzymatic activity was removed by washing with phosphate-buffered saline (PBS), and non-specific antigen was blocked by pre-incubation with PBS containing 0.5% normal goat serum (Vector: S-1000, CA, USA) for type II collagen, normal rabbit serum (Vector: S-5000) for F4/80, or normal horse serum (Vector: S-5000) for PCNA (Proliferating Cell Nuclear Antigen) for 30 min at room temperature. Then, the sections were incubated with rabbit polyclonal anti-mouse type II collagen antibody (1:1000 dilution; Abcam:ab34712, Cambridge, UK), rat anti-mouse F4/80 antibody (1:2000 dilution; AbD Serotec MCA497), or rabbit polyclonal anti-mouse PCNA antibody (1:200 dilution; Abcam:ab53048, Cambridge, UK) at 4 °C overnight. After extensive washing with PBS, the sections were incubated with the secondary antibody of goat poly-biotinylated anti-rabbit IgG (1:200 in dilution; Vector: BA-6100) for type II collagen, with the secondary antibody of rabbit poly-biotinylated anti-rat IgG (1:200 in dilution; Vector: BA-4000) for F4/80, or with the secondary antibody of horse poly-biotinylated anti-rabbit IgG (1:200 in dilution; Vector: BA-4000) for PCNA for 30 min at room temperature. Signals were visualized by Vectastain ABC reagent (Vector: PK-6100) followed by DAB staining. Sections were counter stained with hematoxylin.

2.5. Meniscus morphology, cartilage thickness and chondrocyte density analyses

Morphological parameters are evaluated as described by Matsui et al. [26]. To evaluate the meniscus morphology, cartilage thickness, and chondrocyte density, images of meniscus (as shown in Fig. 3A) were captured, and an area of articular cartilage and adjacent subchondral bone between the anterior and posterior edges of meniscus (217.5 μm in width, as shown in Fig. 2E) was selected. Axio vision software (Carl Zeiss, Jena, Germany) was employed for the evaluation of meniscus morphological parameters (area, length and width) as well as the area of articular cartilage. Average articular cartilage thickness was calculated by dividing the articular cartilage area by the width. Chondrocytes were counted and divided by articular cartilage area to obtain chondrocyte density.

2.6. Cell apoptosis assay

The cell apoptosis assay was performed using a TUNEL detection kit according to the manufacturer's instructions (Takara Shuzo, Kyoto, Japan). Briefly, sections were de-paraffinized in xylene, rehydrated through graded alcohol, and incubated with 15 mg/ml of proteinase K for 15 min at room temperature. Then the sections were immersed in TdT enzyme diluted with Labeling Safe Buffer (provided in the kit) and incubated for 90 min at 37 °C in a humidified chamber. Signals were detected by fluorescence microscopy (Olympus BX 53, Tokyo, Japan). For the staining control, a positive control section which was provided within the kit was used.

2.7. Micro computed tomography (μCT) analysis

To analyze the morphometric changes of epiphyseal trabecular bone structures in the presence or absence of BMP7, left tibiae were harvested and fixed in 70% ethanol. Three dimensional skeletal imaging was captured using a μCT apparatus (Scan Xmate-E090; Comscan Techno Co, Kanagawa, Japan). Epiphyseal trabecular bone volume per tissue volume (BV/TV), trabecular thickness (Tb.Th.), trabecular number (Tb.N) and trabecular spacing

(Tb.Spac.) were calculated using Tri/3D-BONE software (Ratoc System Engineering Co., Tokyo, Japan).

2.8. RNA extraction and quantitative real-time PCR

Articular cartilages and synovial membranes were collected from mice knee joints ($n = 4$) and homogenized in TRIzoll reagent (Ambion, Life Tech, Auckland, USA) with a micro homogenizer (NS-310E) immediately after the harvest. Total RNA was further purified using High Pure RNA Isolation Kit (Roche, Mannheim, Germany). Complementary DNA was synthesized using Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Mannheim, Germany). Amount of each mRNA was quantitated by LightCycler® 480 Real-Time PCR System (Roche, Germany) using FastStart TaqMan®Probe Master Kit (Roche, Mannheim, Germany). Relative mRNA expression level was normalized against endogenous beta-actin as previously described [27]. Sequences of PCR primers were indicated in Supplemental Table 3.

2.9. Statistical analysis

All data are expressed as mean with standard error. The significance of difference was assessed by the Mann–Whitney *U* test with differences considered to be significant at $P < 0.05$.

3. Results

3.1. Elimination of BMP7 expression from the developing limb mesenchyme leads to articular cartilage degeneration after adolescence

Using *Prx1::cre* deleter mice, we successfully eliminated most of the BMP7 expression (more than 95%) in articular cartilage (Fig. 1A), which was also confirmed by whole mount in situ hybridization during the developing limb in our previous report (13). Histological analyses revealed that loss of BMP7 expression from the limb mesenchyme had subtle, if any, effect on articular cartilage formation since we did not observe any significant alteration in safranin-o dye-ability and type II collagen expression in cartilage at 4 weeks of age when the joint formation had completed (Fig. 1B and D). Semi-quantitative evaluation (Mankin's score) confirmed these histological findings (Fig. 1C). In contrast, we observed significant loss of proteoglycan content in articular cartilage of *Bmp7$^{c/c}$;Prx1::cre* mice at 8 weeks (Fig. 2A and B). Since hypertrophic differentiation and cloning (clustering) of articular chondrocytes as well as reduction of cartilage thickness are usually observed during the process of primary OA, we further analyzed the articular cartilage at a cellular level. As shown in Fig. 2C and D, we did not observe any significant progression of hypertrophic differentiation and chondrocyte proliferation, nor did we see a change in the average thickness of the articular cartilage of the femur or the tibia (Fig. 2E and F) at this age. At 24 weeks, we also observed severe reduction in proteoglycan context in articular cartilage matrices in *Bmp7$^{c/c}$;Prx1::cre* mice (Supplemental Fig. 1A), but again did not observe clear progression of articular cartilage degeneration as measured by Mankin's score (compare Supplemental Figs. 1B and 2B), or average thickness of articular cartilage between the genotype (compare Supplemental Figs. 1C and 2F). PCNA staining further supports subtle effects on chondrocyte proliferation in *Bmp7$^{c/c}$;Prx1::cre* mice (Supplemental Fig. 1D and E).

3.2. Morphological and histological features of meniscus and subchondral bone in *Bmp7$^{c/c}$;Prx1::cre* and control mice

Since reduced meniscal function due to injury or malformation is recognized as important risk factors for articular cartilage degeneration [28–31], we performed detailed morphological analyses of meniscus. As shown in Fig. 3A and B, we did not observe any significant difference in size (area) and shape (length and width) of medial meniscus in the absence of BMP7 at 24 weeks. Sagittal sections of anterior region of medial meniscus also revealed that no morphological alteration was observed in *Bmp7$^{c/c}$;Prx1::cre* mice, although safranin-o dye-ability was slightly decreased in the absence of BMP7 (Fig. 3C arrowheads). We did not observe any significant alteration in type II collagen expression in this region (Fig. 3C lower panels).

As subchondral bone sclerosis is associated with the progression of OA [32], we also examined the trabecular bone density in the epiphyseal region of tibia in *Bmp7$^{c/c}$;Prx1::cre* mice at 24 weeks of age. Micro-CT analyses indicated that no significant alteration in trabecular bone structures is observed between the genotype (Fig. 3D and E). In addition, we did not observe osteophyte formation at any stages in the absence of Bmp7 (data not shown).

These data suggest that the cartilage degeneration observed in *Bmp7$^{c/c}$;Prx1::cre* mice is not likely due to developmental (Fig. 1) or structural abnormalities (Fig. 3) of the joint tissues, suggesting a direct role for BMP7 in the maintenance of articular cartilage.

3.3. Loss of BMP7 from the knee joint has no effect on chondrocyte survival, however alters both cartilage matrix protein expression and cartilage matrix degrading enzyme expression

Next, to clarify the functions of endogenous BMP7 in knee joint homeostasis, we further analyzed the articular cartilage at the molecular and cellular levels. As rhBMP7 is a potent anti-apoptotic regulator for articular chondrocytes, and also enhances cartilage matrix production while inhibiting cartilage matrix degradation, we focused on these cellular functions.

As shown in Fig. 4A, we did not observe any TUNEL-positive apoptotic cell in the articular cartilage of both *Bmp7$^{c/c}$;Prx1::cre* mice and control littermates at 8 weeks. In addition, expression of apoptosis-related cysteine proteases, such as caspase3 and caspase8, was not altered in the absence of BMP7 (Fig. 4B). These data suggest that endogenous BMP7 activity may not be involved in the chondrocyte survival. To examine if endogenous BMP7 activity is involved in regulation of the expression of cartilage matrix proteins and/or cartilage matrix degrading enzymes, we extracted total RNA from articular cartilage and performed gene expression analyses. At 8 weeks, we did not observe any significant alteration in expressions of cartilage matrix proteins or matrix degrading enzymes (Fig. 4C). This situation changed as the mice aged, and at 24 weeks, we observed a significant decrease in aggrecan gene expression concomitant with an increase in matrix metalloproteinase 13 expression (Fig. 4D). These data suggest that endogenous BMP7 activity may act as an anabolic and anti-catabolic regulator for mature articular cartilage.

3.4. Synovial auto-inflammation and hyperplasia are observed in the absence of endogenous BMP7

Synovial inflammation and synovial hyperplasia are usually associated with the progression of OA [33]. So, we examined the synovial membranes of mice lacking BMP7 for potential changes. As shown in Fig. 5A, the cellularity of the synovial membrane was quite similar in the presence or absence of BMP7 in young mice (4 weeks of age; higher magnification in the middle panels), although there was a slightly increased accumulation of F4/80-positive macrophages in the absence of BMP7 (lower panels). At 8 and 24 weeks of age, we observed extensive synovial hyperplasia (middle panels) and significant numbers of F4/80 positive-macrophage infiltrating into the synovial membrane in *Bmp7$^{c/c}$;Prx1::cre* mice (Fig. 5B), although the severity of synovial

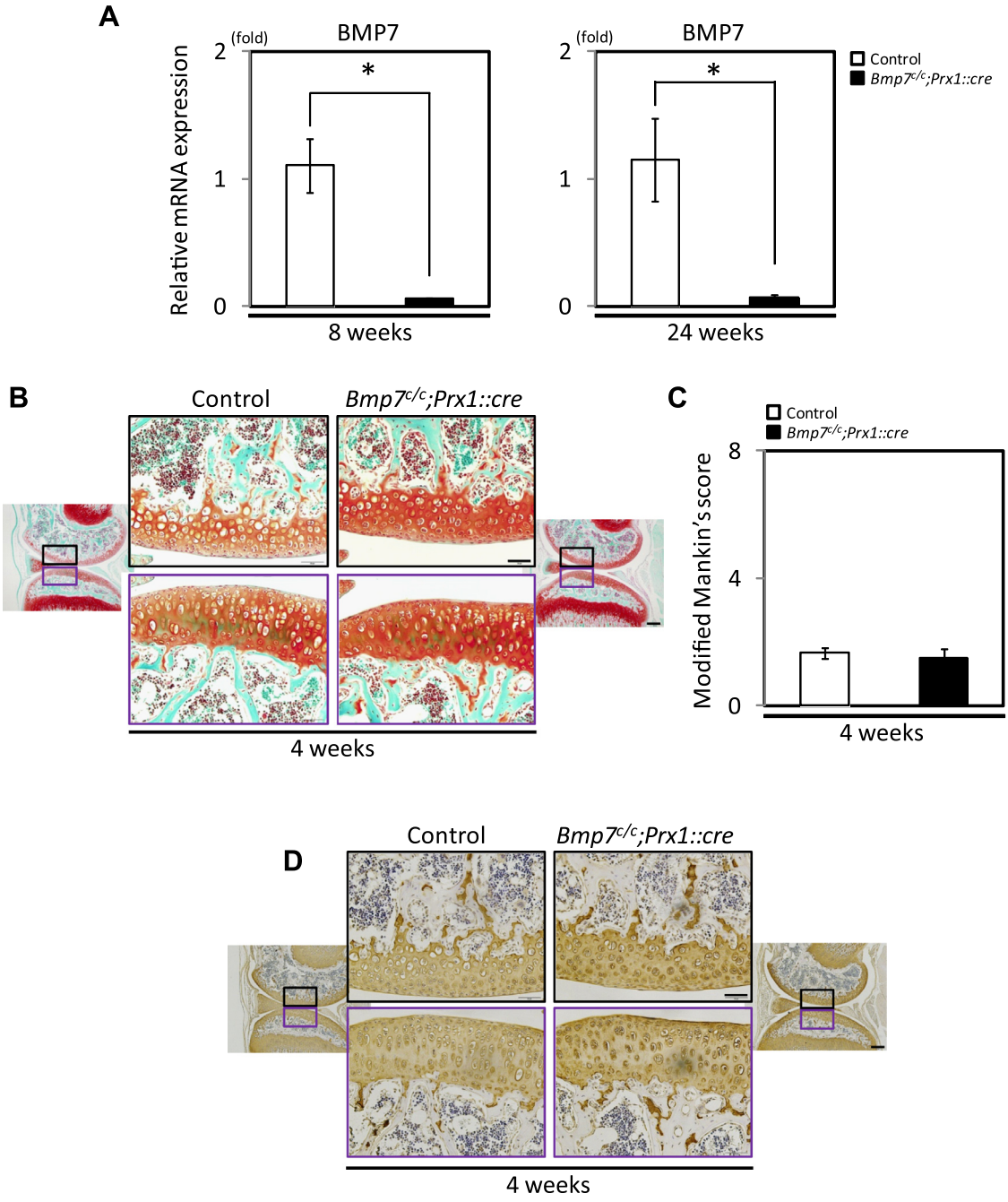

Fig. 1. Elimination of BMP7 expression from the developing limb mesenchyme did not affect knee joint formation. (A) BMP7 gene expression in articular cartilage at 8 and 24 weeks of age quantitated by Q-PCR. Values were represented by mean ± SE ($n = 4$). (B) Safranin-O staining of sagittal sections of knee joint at 4 weeks of age. Sections were counterstained by fast green. Upper; femur, lower; tibia. Boxed area in low-power field was indicated in high-power field. A scale bar in high-power field (20×) indicates 50 μm, in low-power field (4×) indicates 200 μm. (C) Semi-quantitative evaluation of articular cartilage degeneration by modified Mankin's scoring system. Values were represented as mean ± SE ($n = 3$). (D) Type II collagen expression in knee joint at 4 weeks of age. Boxed area in low-power field was indicated in high-power field. A scale bar in high-power field (20×) indicates 50 μm, in low-power field (4×) indicates 200 μm. Asterisks indicate $P < 0.05$.

hyperplasia seemed less at 24 weeks than 8 weeks (Fig. 5C). These histological observations were confirmed using a semi-quantitative synovitis scoring system; we observed significant increases in synovitis scores in *Bmp7$^{c/c}$;Prx1::cre* mice at both 8 and 24 weeks (Fig. 5D–F). Gene expression analyses indicated that among the proinflammatory cytokines present, Activin A expression was significantly increased in synovial membrane from *Bmp7$^{c/c}$;Prx1::cre* mice at 24 weeks while we observed comparable levels of IL-1β between the genotype (Fig. 5G). These data suggest that endogenous BMP7 has anti-inflammatory effects that dampen the synovial hyperplasia often seen in aging mice.

4. Discussion

Our study is the first to describe a physiological role for locally produced BMP7 in the maintenance of synovial joint homeostasis in adults. These results are in line with the phenotype of joint specific Alk3 conditional knockout mice, where joint formation occurs but the joint structures gradually decline with age [15]. The joint phenotype we observed in the absence of BMP7 is much milder than that reported for loss of Alk3, consistent with the knowledge that other osteogenic BMPs such as BMP2 and BMP4, are also ligands for Alk3, and are also present in articular cartilage

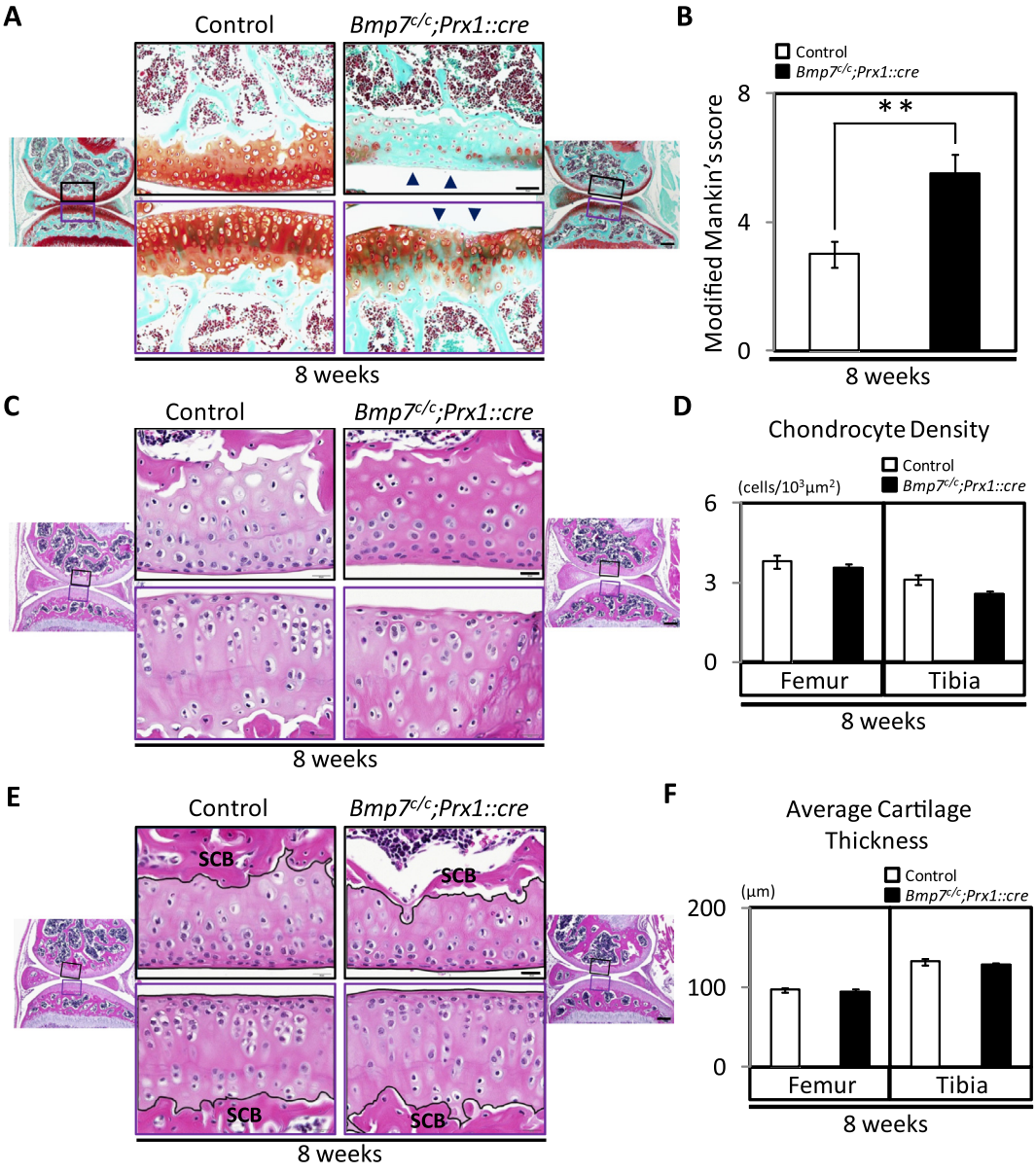

Fig. 2. Elimination of BMP7 expression from the developing limb mesenchyme leads to articular cartilage degeneration at 8 weeks of age. (A) Safranin-O staining of sagittal sections of knee joint. Sections were counterstained by fast green. Upper; femur, lower; tibia. Arrowheads indicate the region where proteoglycan contents were decreased. Boxed area in low-power field was indicated in high-power field. A scale bar in high-power field (20×) indicates 50 μm, in low-power field (4×) indicates 200 μm. (B) Semi quantitative evaluation of articular cartilage degeneration by modified Mankin's scoring system. Values were represented as mean ± SE ($n = 7$). (C) H/E staining of sagittal sections of knee joint. Boxed area in low-power field was indicated in high-power field. A scale bar in high-power field (40×) indicates 20 μm, in low-power field (4×) indicates 200 μm. (D) Chondrocyte density in articular cartilage. Number of chondrocytes in the high-power field (boxed area in the low-power field) was counted and plotted. Values were represented as mean ± SE ($n = 7$). (E, F) Area of articular cartilage was contoured as indicated in panel E and quantitated by Zeiss Axio Vision. A scale bar in high-power field (40×) indicates 20 μm, in low-power field (4×) indicates 200 μm. Average cartilage thickness was calculated as described in materials and methods. Values were represented as mean ± SE ($n = 7$). Double asterisks indicate $P < 0.01$. SCB; subchondral bone.

(Supplementary Fig. 3). In our previous studies, we showed that loss of BMP2 from the developing limb (*Bmp2*$^{c/c}$;*Prx1::cre*), did not affect joint formation during embryogenesis, but caused a delay in secondary ossification center formation and reduced type II collagen expression from articular cartilage by 2 weeks of age [11]. As loss of BMP7 did not result in changes in type II collagen expression (Figs. 1D, 4C and D and Supplemental Fig. 2), our data suggest that each BMP molecule may have distinct function in cartilage based on their temporal and spatial expression patterns.

Our histological analyses indicated that cartilage morphology and thickness were quite normal in the absence of BMP7, as was the morphology of the medial meniscus and the underlying subchondral bone, suggesting that along with endogenous BMP7 activity, other factors are also necessary for the progression of OA. This idea is supported by epidemiological analyses of OA that show it to be a multifactorial diseases affected by diverse factors such as age, hormone status, body weight, heredity, hypertension, and abnormal mechanical stress [34–39]. We believe that BMP7 status is an additional component to OA susceptibility and expect that genetic/epi-genetic analyses of BMP7 gene locus may give us important information to predict the prevalence of OA.

In our study, endogenous BMP7 activity was not directly involved in the proliferation, hypertrophic differentiation, and survival of articular chondrocytes, findings that are in stark contrast to data reported previously by other groups [17,40–42]. We predict

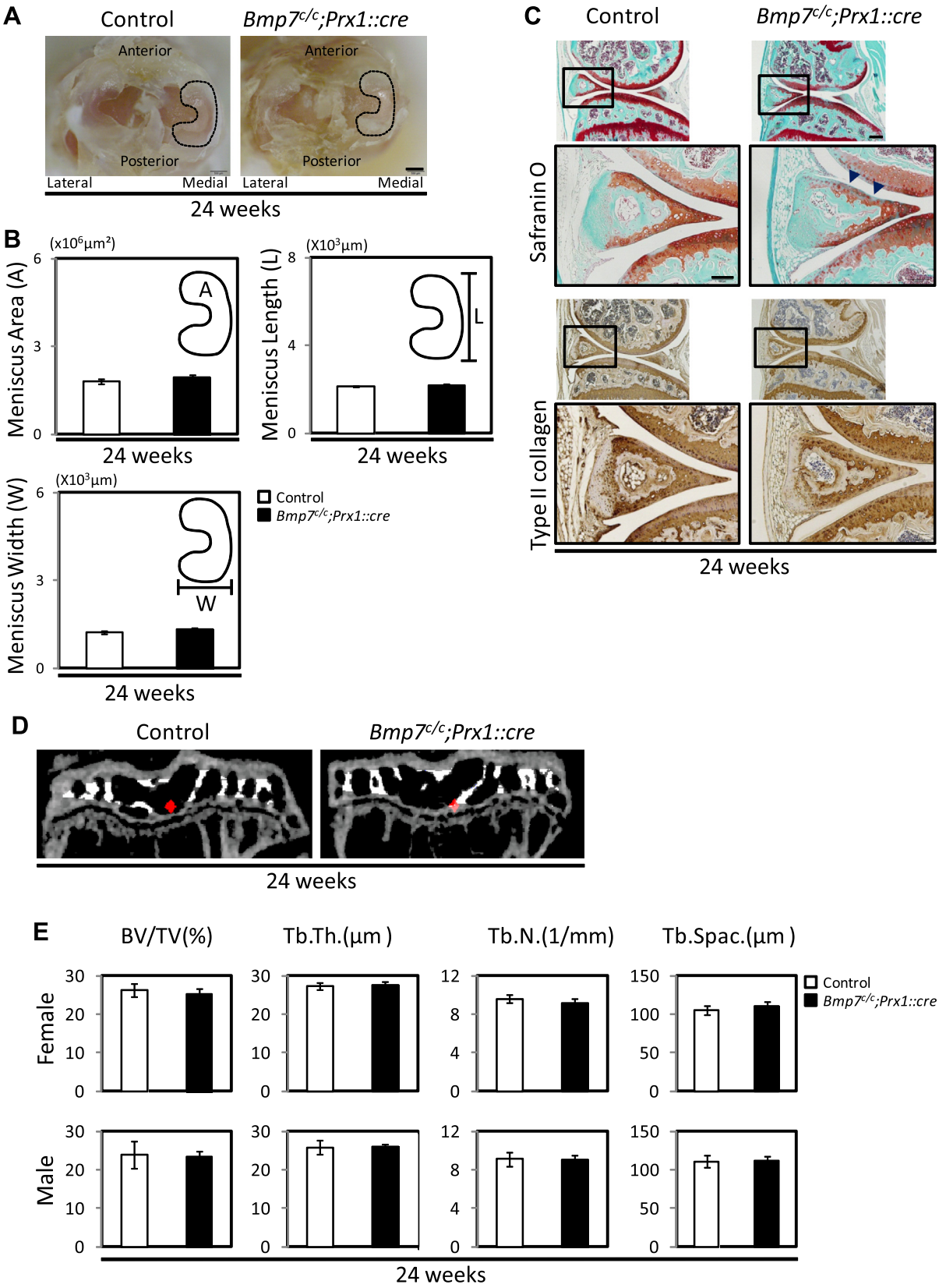

Fig. 3. Histomorphometrical analysis of medial meniscus and epiphyseal trabecular bone of *Bmp7$^{c/c}$;Prx1::cre* mice at 24 weeks of age. (A) Superior view of tibial plateau of control and *Bmp7$^{c/c}$;Prx1::cre* mice at 24 weeks of age. Medial meniscus was contoured by dotted lines. A scale bar indicates 500 μm. (B) Morphological evaluation of medial meniscus. Area, length and width of medial meniscus were measured based on the macroscopic observation ($n = 9$). (C) Histological analysis of anterior medial meniscus. Upper panels; safranin-O staining of sagittal sections of anterior medial meniscus. Sections were counterstained by fast green. Boxed areas in low-power field (4×) were indicated in high-power field (10×). Arrowheads indicated reduced safranin-o dye-ability in *Bmp7$^{c/c}$;Prx1::cre*. Lower panels; type II collagen immuno-staining. A scale bar in high-power field (10×) indicates 100 μm, in low-power field (4×) indicates 200 μm. (D, E) Bone histomorphometrical analysis of tibia epiphysis by μCT. Histomorphometrical parameters in trabecular bone area highlighted by white in panel D was quantitated and plotted in panel E. Values represent mean ± SE ($n = 9$).

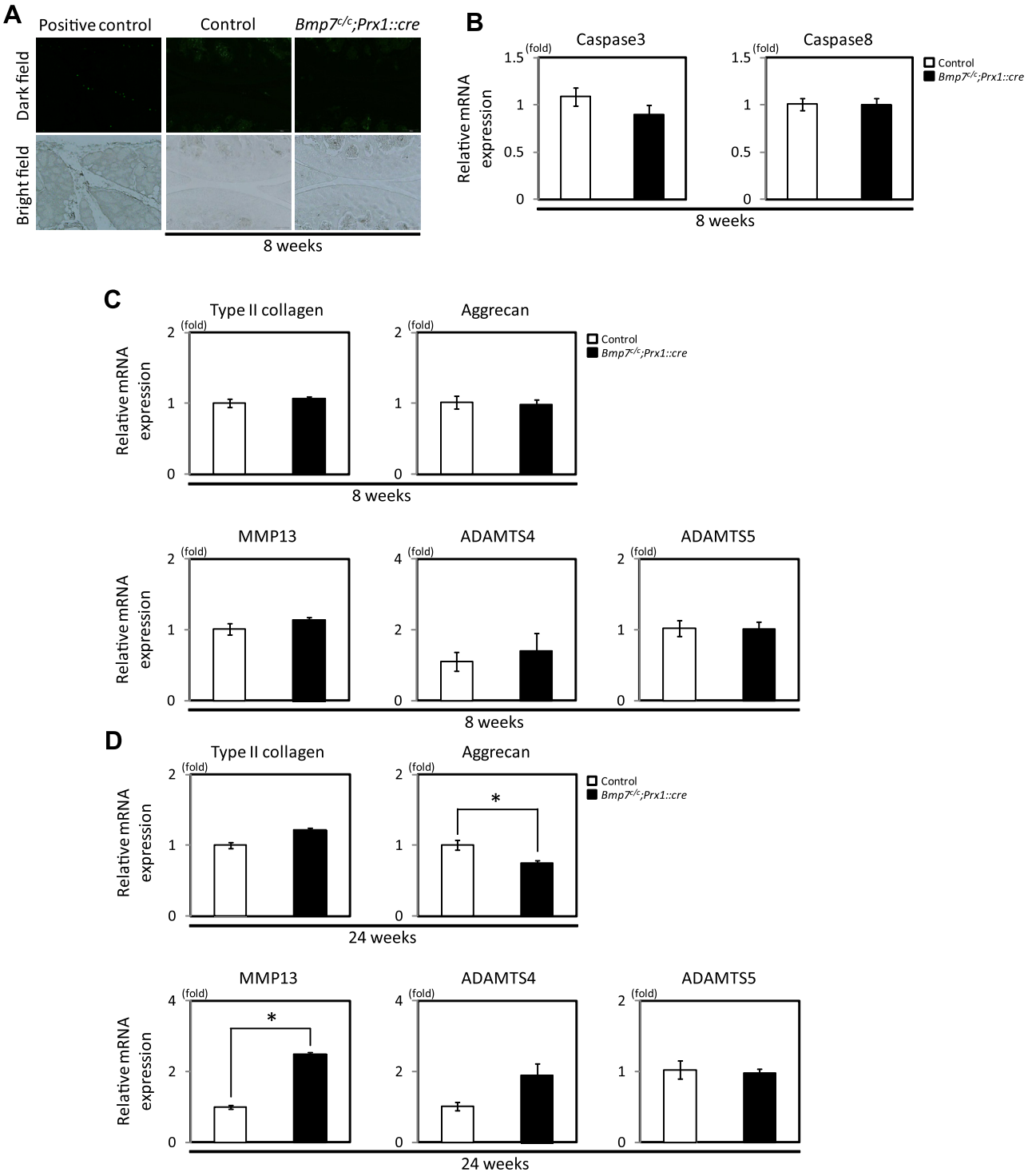

Fig. 4. Effects of endogenous BMP7 on chondrocyte survival and gene expression profiles. (A) TUNEL staining of articular cartilage at 8 weeks. Cells with green fluorescence undergo death by apoptosis. Scale bar in pictures (10×) indicates 100 μm (B) Apoptosis-related gene expression in articular cartilage at 8 weeks ($n = 4$). (C, D) Anabolic- and catabolic-gene expressions in articular cartilage at 8 and 24 weeks ($n = 4$). Values represent mean ± SE. Asterisk indicates $P < 0.05$.

that this difference in findings was due to the difference in dosage between endogenously expressed BMP7 and the amount used for intra-articular injections.

Our data are consistent with a role for rhBMP7 in both anabolic and anti-catabolic functions in articular cartilage [43,44]. It is also possible that cells other than articular chondrocytes may be targets for BMP7 in the adult synovial joint. One interesting candidate is synovial macrophage, as this cell type is observed in higher abundance in 8 week-old *Bmp7$^{c/c}$;Prx1::cre* mice. These cells may also be the source of the proinflammatory cytokine, Activin A, whose expression was significantly increased in the absence of BMP7. Since reduction of articular cartilage proteoglycan content was observed at the same time as higher Activin A production, we hypothesize that the synovial inflammation observed in *Bmp7$^{c/c}$;Prx1::cre* mice is due to enhanced auto-inflammatory responses.

In summary, we showed that the absence of BMP7 in the knee joint results in significant loss of proteoglycan from

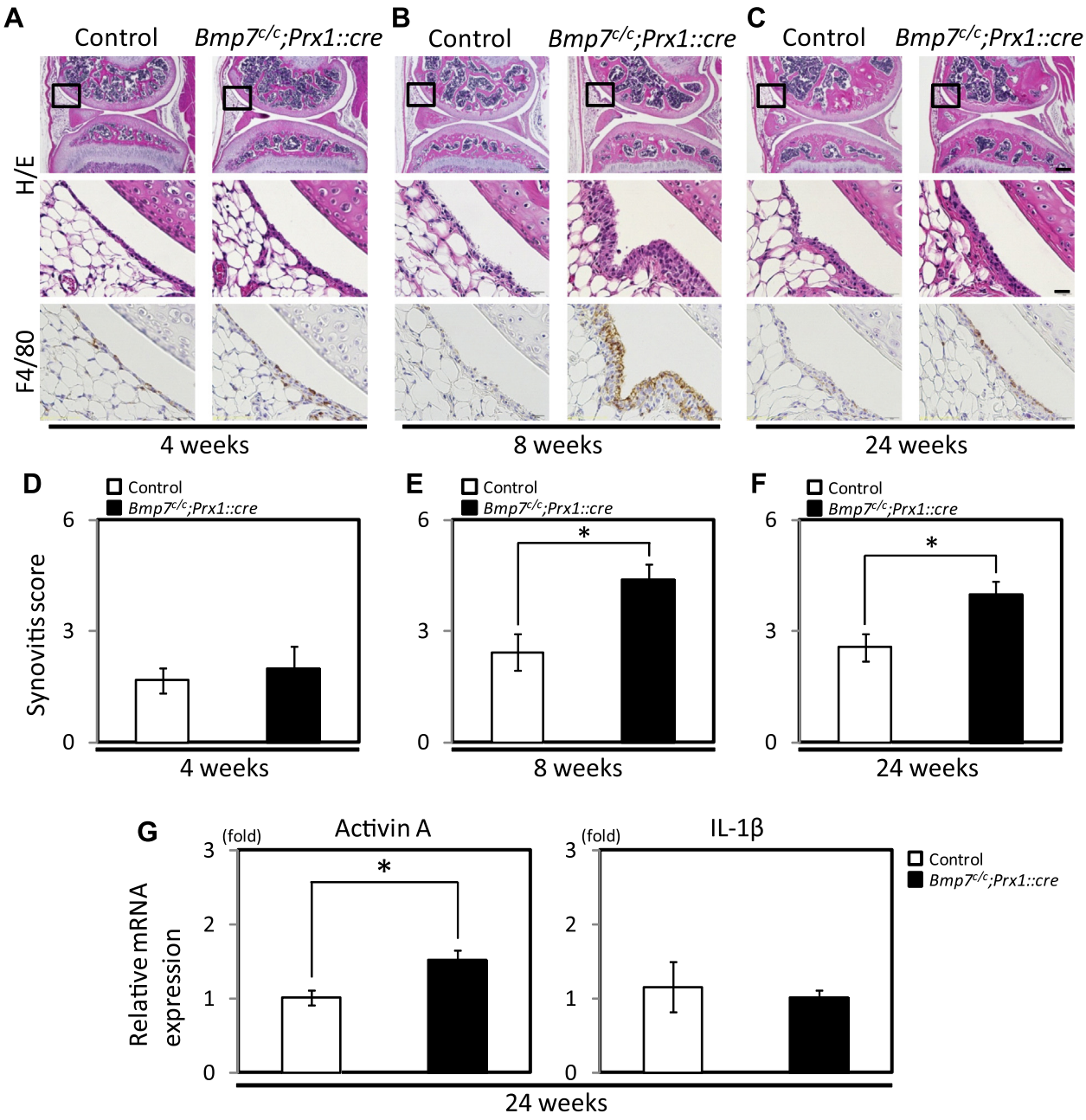

Fig. 5. Elimination of BMP7 expression from the developing limb mesenchyme leads to auto-inflammation in synovial membrane after 8 weeks. (A, B, C) H/E staining of sagittal sections of knee joint at 4, 8, and 24 weeks. Boxed areas in upper panels were magnified and indicated in the middle panels. Immunohistochemical staining of synovial membrane by mouse macrophage-specific f4/80 antibody (lower panels). A scale bar in high power field (40×) indicates 20 μm, in low power field (4×) indicates 200 μm (D, E, F) Semi-quantitative analyses of the severity of synovial inflammation by synovitis scoring system. (4 weeks; $n = 3$, 8 weeks; $n = 7$, 24 weeks; $n = 9$). (G) Activin and IL-1β expression in synovial membrane of *Bmp7$^{c/c}$;Prx1::cre* mice and control littermates at 24 weeks of age quantitated by Q-PCR. Values represent mean ± SE. Asterisk indicates $P < 0.05$. ($n = 4$).

articular cartilage and also correlates with an increased synovial auto-inflammatory response in adult mice. Our results are consistent with a role for endogenous BMP7 activity in synovial joint homeostasis. Since rhBMP7 is currently available for clinical use, we believe that it may be beneficial to supplement endogenous BMP7 with rhBMP7 to prevent or slow the development of primary OA.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2015.04.004.

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