

RESEARCH ARTICLE

Alpha 5 Integrin Mediates Osteoarthritic Changes in Mouse Knee Joints

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Abstract

Osteoarthritis (OA) is one of most common skeletal disorders and can affect synovial joints such as knee and ankle joints. $\alpha 5$ integrin, a major fibronectin receptor, is expressed in articular cartilage and has been demonstrated to play roles in synovial joint development and in the regulation of chondrocyte survival and matrix degradation in articular cartilage. We hypothesized that $\alpha 5$ integrin signaling is involved in pathogenesis of OA. To test this, we generated compound mice that conditionally ablate $\alpha 5$ integrin in the synovial joints using the *Gdf5Cre* system. The compound mice were born normally and had an overall appearance similar to the control mice. However, when the mutant mice received the OA surgery, they showed stronger resistance to osteoarthritic changes than the control. Specifically the mutant knee joints presented lower levels of cartilage matrix and structure loss and synovial changes and showed stronger biomechanical properties than the control knee joints. These findings indicate that $\alpha 5$ integrin may not be essential for synovial joint development but play a causative role in induction of osteoarthritic changes.

Introduction

Osteoarthritis (OA) is one of the most common skeletal diseases and involves pathological changes in synovial joint components such as articular cartilage and synovium. Pathological changes seen in articular cartilage in OA include irregularity and loss of articular surface, loss of proteoglycan matrix and alterations of collagen fibrils and fibers, and cleft and erosion of articular cartilage [1]. These changes result from many cellular events including cytoskeletal changes, proliferation, matrix synthesis and degradation, cell senescence and apoptosis, as well as hypertrophy [2]. In addition to alterations of cartilage structure and function, thickening of

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subchondral bone and ectopic bone formation (osteophyte) occur in OA [3, 4]. Synovial changes, including synovitis and hyperplasia of synovial cells, are found in synovial joints from the early stages of osteoarthritis, and have been studied as causative events and therapeutic targets of this condition [5, 6]. Continuous and extensive efforts have been made to understand the biology of joint homeostasis and the pathogenesis of OA for the development of therapy for this disease, though no effective disease-modifying osteoarthritis drug has been discovered.

Alpha 5 integrin ($\alpha 5$ integrin) is a fibronectin receptor that mediates a variety of biological phenomena including mesoderm induction, vascular development, and neural crest development [7–10]. Human articular chondrocytes express $\alpha 5\beta 1$ together with many other heterodimers of integrins such as $\alpha 1\beta 1$, $\alpha \nu\beta 5$, $\alpha \nu\beta 3$ and $\alpha 3\beta 1$ [11, 12]. Among these integrins, $\alpha 5\beta 1$ has been demonstrated to play roles in synovial joint development [13] and in the regulation of chondrocyte survival, matrix degradation, and expression of cytokines and non-cartilaginous collagens [14–18]. Based on these findings, we hypothesized that $\alpha 5$ integrin signaling is involved in pathogenesis of OA. To test this hypothesis, we generated conditional knockout mice lacking $\alpha 5$ integrin expression specifically in synovial joints through use of the GDF5Cre system [19, 20] and analyzed the pathological and biomechanical changes of knee joints after OA surgery. The findings suggest that $\alpha 5$ integrin mediates the progression of OA, possibly targeting synovial cells.

Materials and Methods

Mice

All animal experiment procedures were approved by the Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia. The Institute maintains veterinary personnel who supervise our work and answer any question we may have related to the use and care of vertebrate animals. When pain/distress are observed, the animals will be treated with Buprenex (0.1–2.0 mg/kg), as well as crushed or wet food. If pain or distress continues, we will euthanize the mouse regardless of the scheduled endpoints. The criteria that determine discomfort/distress/pain are any three of the following signs: Abnormal posture, slow, careful or abnormal (waddling) gait, low activity, slow eating, cowering or vocalizing while handling, change in eye or coat appearance and weight loss. We strictly follow the American Veterinary Medical Association Panel on Euthanasia for adult mice and NIH Euthanasia Guidelines for mouse embryos and neonates. Specifically, mice older than 14 days will be euthanized by CO₂ inhalation. We will assure euthanasia by one of the following criteria: Cervical dislocation after no response to tail or toe pinch, no respiration or heartbeat after thirty seconds continuous monitoring, or rigor mortis. We will give anesthesia (isoflurane inhalation) to mouse embryos older than E15 and neonates up to 14 days prior to decapitation. Mice will be anesthetized by inhalation of 1–5% of isoflurane during OA surgery and tail clipping for genotyping.

$\alpha 5$ integrin was conditionally knocked out of the synovial joints by generation of the triple compound transgenic mouse that encodes Cre under the control Gdf5 expression (*Gdf5Cre*) [19], *flox $\alpha 5$ integrin* [9] and $\alpha 5$ null alleles [8]. First we mated the *Gdf5Cre* mouse with the $\alpha 5$ null mouse. The resulting double-het (*Gdf5Cre*; $\alpha 5$ null/*wt*) mouse was mated with homozygous floxed $\alpha 5$ integrin mouse (*$\alpha 5$ fl/fl*) to generate the triple compound mouse (*Gdf5Cre*; $\alpha 5$ null/*fl*), hereafter called $\alpha 5$ CKO). The genotyping for the *Gdf5Cre*, $\alpha 5$ null and *flox $\alpha 5$* alleles and the excised allele of the *floxed $\alpha 5$* have been described elsewhere [9]. The *Gdf5Cre*; $\alpha 5$ fl/*wt*, $\alpha 5$ fl/*null* or $\alpha 5$ fl/*wt* were used as the control since they do not show significant differences in OA phenotype after OA surgery. To monitor the fate of Gdf5-lineage cells, we generated the *Gdf5Cre*;Rosa-GFP compound mouse. To determine localization of $\alpha 5$ integrin, we used C57BL/6j (Jackson Lab, Bar Harbor, ME, USA) mice.

OA surgery

Under anesthesia, we transected the medial collateral ligament and removed the medial meniscus in the right knee in both $\alpha 5$ CKO and control littermate male mice ($n = 9$ /group) at 3 months of age as described previously [21]. Knees were collected and examined by histological and histochemical means 1 month after surgery.

Histology and immunostaining

The knee joints were fixed with 10% formalin or 4% paraformaldehyde overnight and decalcified with EDTA for 2 weeks. The paraffin sections were stained with hematoxylin and eosin or Safranin O staining. To detect apoptotic cells, we performed TUNEL staining using the Apop-Tag peroxidase *in situ* apoptosis detection kit (EMD Millipore, Billerica, MA, USA) and the In Situ Cell Death Detection Kit, Fluorescein (Roche, Branford, CT, USA). For immunohistochemical staining for collagen 10, MMP13, VDIPEN, CD31, and GFP, the paraffin sections were pre-treated with 0.1% pepsin (collagen 10 and MMP13: 10 min at 37°C) and/or 10 mM sodium citrate (MMP13, VDIPEN, CD31 and GFP: 10 min at 95°C) and then incubated with primary antibodies (collagen 10: Cosmo Bio USA (Carisbad, CA, USA), 1:1000; MMP13: Abcam (Cambridge, UK), 1:250; VDIPEN (gift from Dr. J. Mort (Shriners Hospital), 1:1000; CD31: Santa Cruz (Dallas, TX), 1:250; GFP: Cell Signaling (Danvers, MA, USA), 1:250) overnight at 4°C. The antibodies were visualized by incubation with appropriate biotinylated antibodies (Vector Lab, Burlingame, CA, USA) followed by the color development method using the ImmPACT NovaRED Peroxidase Substrate kit (Vector Lab). The sections were counterstained with Fast Green. For immunofluorescence staining for $\alpha 5$ integrin, the frozen sections were incubated with the primary antibody against $\alpha 5$ integrin (BD BioSciences, San Jose, CA, USA) followed by incubation with the biotinylated antibodies (Vector Lab) and Texas-Red NeutrAvidin (Thermo Fisher Scientific, Waltham, MA, USA). The nuclei were stained with DAPI. Staining data were examined with a Nikon Eclipse TE400 equipped with SPOT 5.0 Advanced software (Diagnostic Instruments Inc., Sterling Heights, MI, USA), a Nikon Eclipse TE2000 microscope equipped with an Image-Pro 7.0 software (Media Cyberkinetics, Rockville, MD, USA), or Leica TCS LSI confocal microscope system (Buffalo Grove, IL, USA). The ratio of TUNEL positive cells in sections was determined using images captured with Image-Pro 7.0 software. The number of fluorescence-labeled cells was divided by the total number of cells across all articular cartilage zones ($n = 6$ /group).

Histological evaluation of osteoarthritic changes

The slides of knee joints were stained with hematoxylin and eosin or Safranin O in increments of five slides and inspected by two independent researchers. The extent of changes in medial tibial plateau and medial femoral condyle of articular cartilage was evaluated using Mankin and OARSI scoring systems [22]. The extent of changes in the synovium was evaluated following the scale from a previous report [23]: 0, normal (synovial lining 1–3 cells thick); 1, mild inflammation (synovial lining 4 or 5 cells thick, increased cellularity); 2, moderate inflammation (synovial lining 6–8 cells thick and/or increase in cellularity); and 3, severe inflammation (synovial lining >9 cells thick and dense cellularity). The synovium adjacent to the meniscus toward the femur and the tibia were examined separately (4–5 sections/mouse).

Nanoindentation

A separate group of mice were used for atomic force microscopy (AFM)-based nanoindentation, following our previously established procedures [24].

Briefly, the condyles from hind knees were dissected immediately after euthanasia by carbon dioxide, and mounted on steel AFM sample disks. Through this process, the joints were kept in sterile phosphate buffered saline (PBS) with protease inhibitors to minimize post-mortem degradation. Nanoindentation was performed on the surfaces of medial condyle cartilage using a borosilicate microspherical tip ($R \approx 5 \mu\text{m}$, nominal spring constant $k \approx 7.4 \text{ N/m}$, AIO-TL tip C, NanoAndMore) and a Dimension Icon AFM (BrukerNano). On each condyle, at least 10–15 different locations were tested up to an indentation depth of $\sim 1 \mu\text{m}$ at an approximately $10 \mu\text{m/s}$ indentation rate. The effective modulus, E_{ind} , was calculated by fitting the loading portion of each indentation force-depth curve to the elastic Hertz model via least squares linear regression, $F = 4E_{ind}R^{1/2}D^{3/2}/[3(1 - \nu^2)]$, where R is the tip radius, and ν is Poisson's ratio (~ 0.1 for cartilage) [25].

Statistics

Student's t-tests or two-way factorial ANOVA followed by Bonferroni post-hoc multiple comparison tests were used to identify the differences. The threshold for significance for all tests was set as $p < 0.05$.

Results

1. Ablation of $\alpha 5$ integrin in synovial joints in mice

The *GDF5Cre; $\alpha 5$ fl/-* mice were born normally and had an overall appearance similar to the control mice. Histological analysis of the knee joints of the *GDF5Cre; $\alpha 5$ fl/-* mice did not show significant abnormalities in the structure of synovial joints including articular cartilage (Fig 1A vs 1D) and synovium (Fig 1G vs 1J) compared to the control mice. In the control mice, expression of $\alpha 5$ integrin was strongly expressed in articular cartilage at P0 (S1 Fig) and gradually decreased, but remained in the articular surface (Fig 1B and S1 Fig) at 4 weeks of age. $\alpha 5$ expression of the synovium was very strong (Fig 1H). In contrast, the $\alpha 5$ CKO mouse did not show positive staining for $\alpha 5$ integrin in articular cartilage (Fig 1E and S1 Fig) and presented much weaker staining in synovium (Fig 1K) while the perichondrium adjacent to growth plate showed $\alpha 5$ staining similar to the control mice (S1 Fig).

2. Analyses of osteoarthritic changes in the $\alpha 5$ CKO articular cartilage

Next we compared the osteoarthritic changes between the $\alpha 5$ CKO and the control mice after OA surgery. Interestingly, we observed significantly milder histological osteoarthritic changes in the tibial medial plateau and femoral medial condyle of the $\alpha 5$ CKO mice (Fig 2B, 2D and 2F) than those in the control mice (Fig 2A, 2C and 2E). Both matrix loss and structural changes were less evident in the $\alpha 5$ CKO articular cartilage compared to the control articular cartilage. Histological evaluation by Mankin and OARSI score methods demonstrated that the $\alpha 5$ CKO articular cartilage in the OA surgery side showed significantly lower values than the control articular cartilage (Fig 2G and 2H).

To examine whether milder histological changes in the $\alpha 5$ CKO articular cartilage was related to the changes in cartilage biomechanical properties, we performed AFM-nanoindentation assessment on the femoral condyles with or without surgery. The $\alpha 5$ CKO articular cartilage with OA surgery showed a significantly higher effective indentation modulus (E_{ind}) than the control (Fig 3, $p = 0.0482$). In addition, the average of the E_{ind} of the $\alpha 5$ CKO articular cartilage without surgery was found to be marginally higher than the control ($p = 0.0563$, Fig 3).

To gain more insights into understanding pathological changes in articular cartilage in the $\alpha 5$ CKO mice, we examined typical molecular changes associated with OA. The $\alpha 5$ CKO group

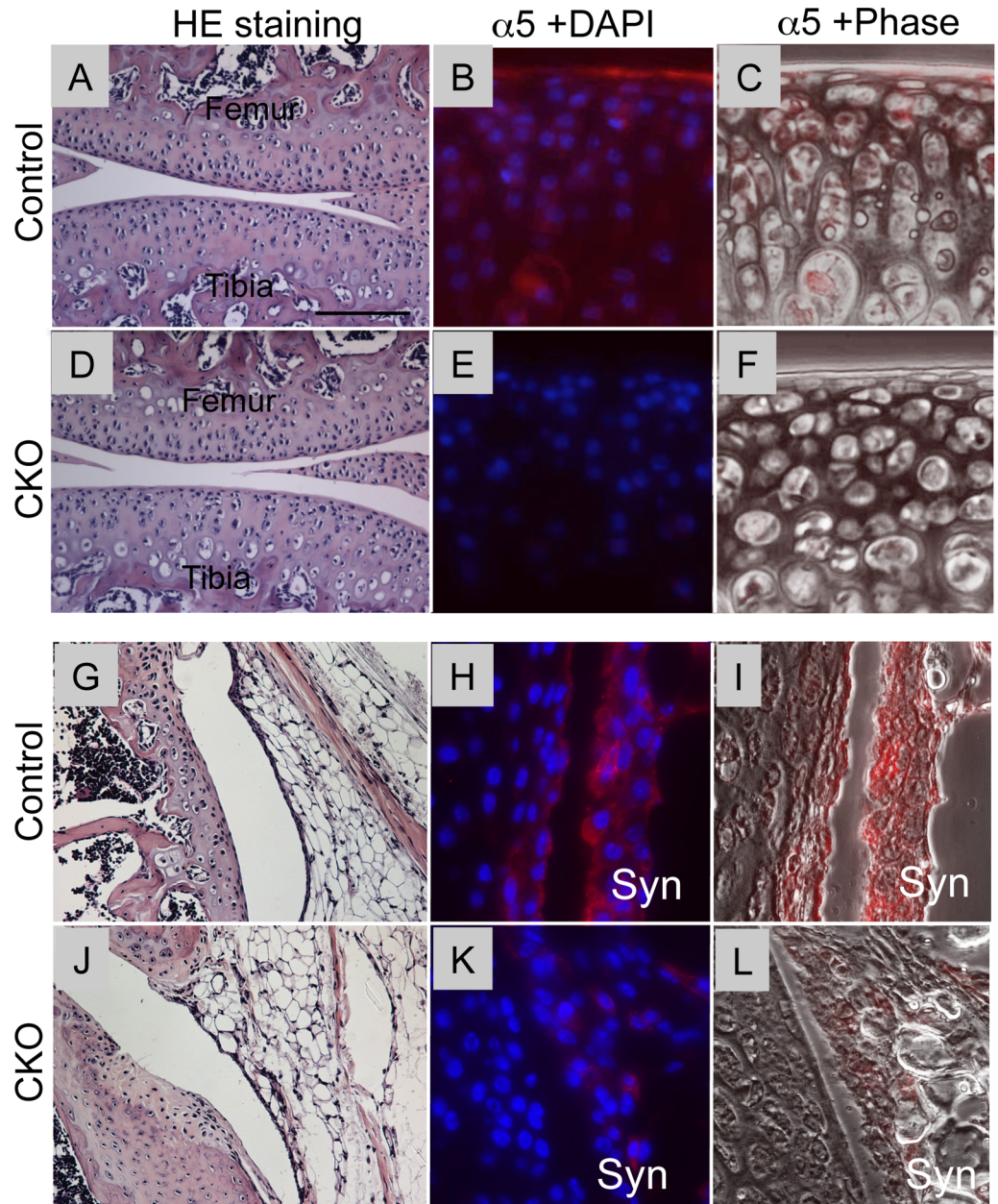


Fig 1. Histology and expression of $\alpha 5$ integrin in synovial joints in control and $\alpha 5$ CKO mice. The knee joints were harvested from control (A-C and G-I) and $\alpha 5$ CKO (D-F and J-L) at 4 weeks of age and subjected to histological inspection (A, D, G and J) and immunofluorescence staining of $\alpha 5$ integrin (B, E, H and K). A, D, G and J, the images of articular cartilage (A and D) and synovium (G and J) stained with hematoxylin-eosin. B, E, H and K, Superimposed images of $\alpha 5$ integrin staining (red) with DAPI nuclear staining (blue). C, F, I and L, Superimposed images of $\alpha 5$ integrin staining with the phase contrast images. Bar, 250 μ m for A, D, G and J; 40 μ m for B, C, E, F, H, I, K and L.

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(Fig 4A and 4C) contained a smaller number of TUNEL-positive cells than the control group (Fig 4B and 4D) (S2 Fig, TUNEL). Semi-quantitative analysis for number of TUNEL positive cells confirmed fewer TUNEL-positive cells in the $\alpha 5$ CKO group than the control group (Fig 4E). Expression of collagen 10, a hypertrophic marker, was found in the eroded deep zone of the control articular cartilage (S2E and S2F Fig, arrows). The $\alpha 5$ CKO articular cartilage had

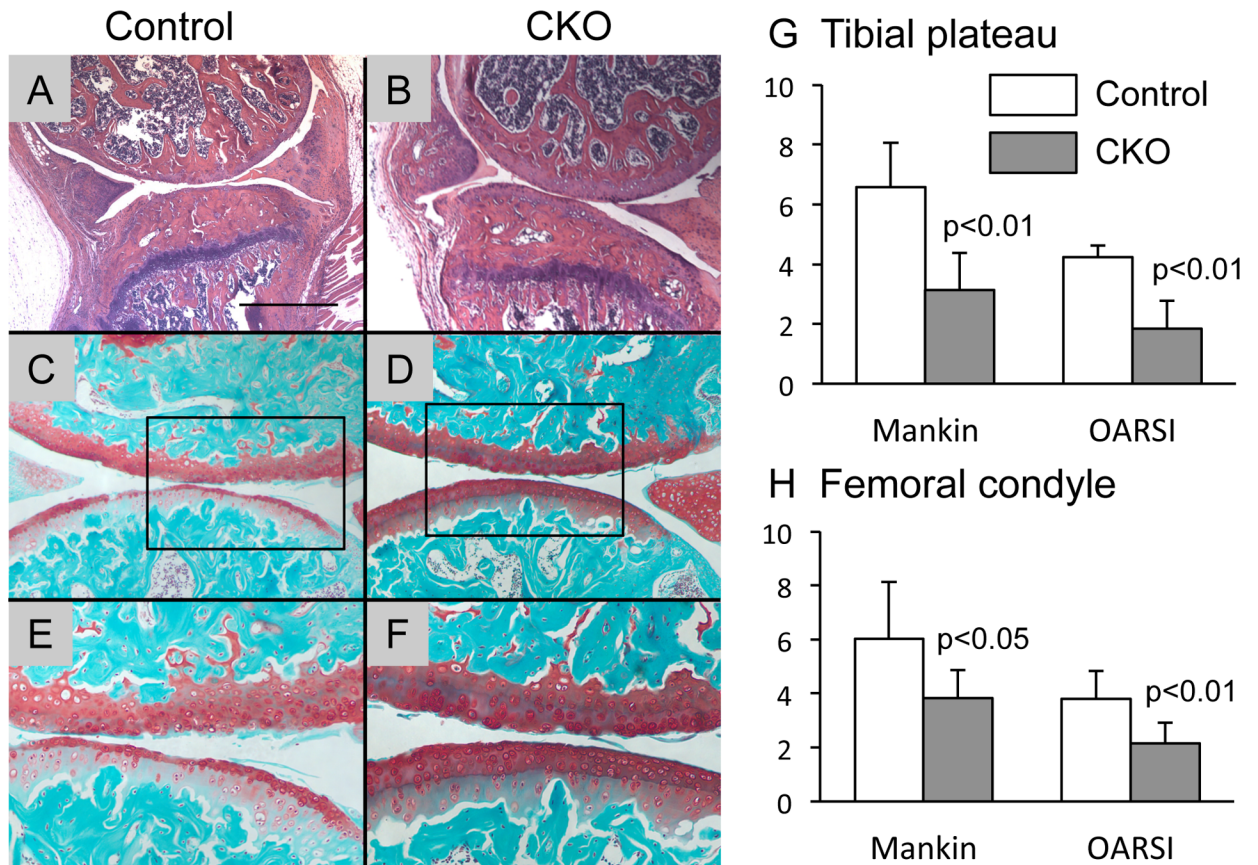


Fig 2. Osteoarthritic changes in control and $\alpha 5$ CKO mice. The knee joints were harvested from control (A, C and E) and $\alpha 5$ CKO (B, D and F) 4 weeks after OA surgery and subjected to histological inspection. A-F, representative images of the medial femoral condyle and the medial tibial plateau stained with hematoxylin-eosin (A and B) and Safranin O (C-F). E and F are magnified images of the box of C and D, respectively. Bar, 625 μ m for A and B; 250 μ m for C and D; 125 μ m for E and F. G and H, Mankin and OARSI scores of the medial tibial plateau (G) and the medial femoral condyle (H). The graphs represent average and SD, n = 9/group.

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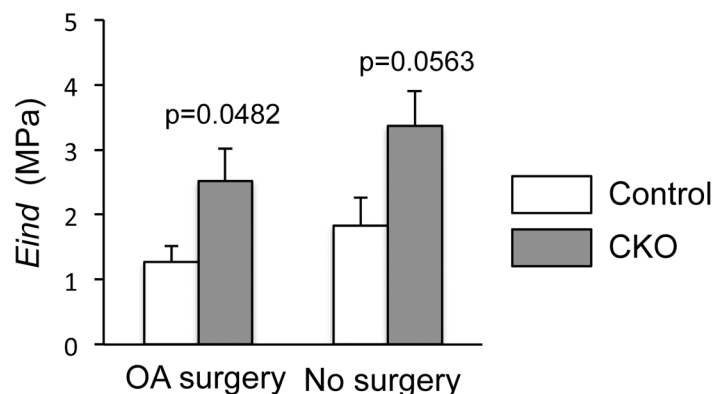


Fig 3. Biomechanical properties in the articular cartilage in control and $\alpha 5$ CKO mice. The knee joints were harvested from control and $\alpha 5$ CKO mice 4 weeks after OA surgery (OA surgery) and without surgery (No surgery). The femoral condyle was subjected to AFM-based nanoindentation assessment. The graph represents average and SE, n = 5/group. E_{ind} , Effective indentation module calculated from Hertz model.

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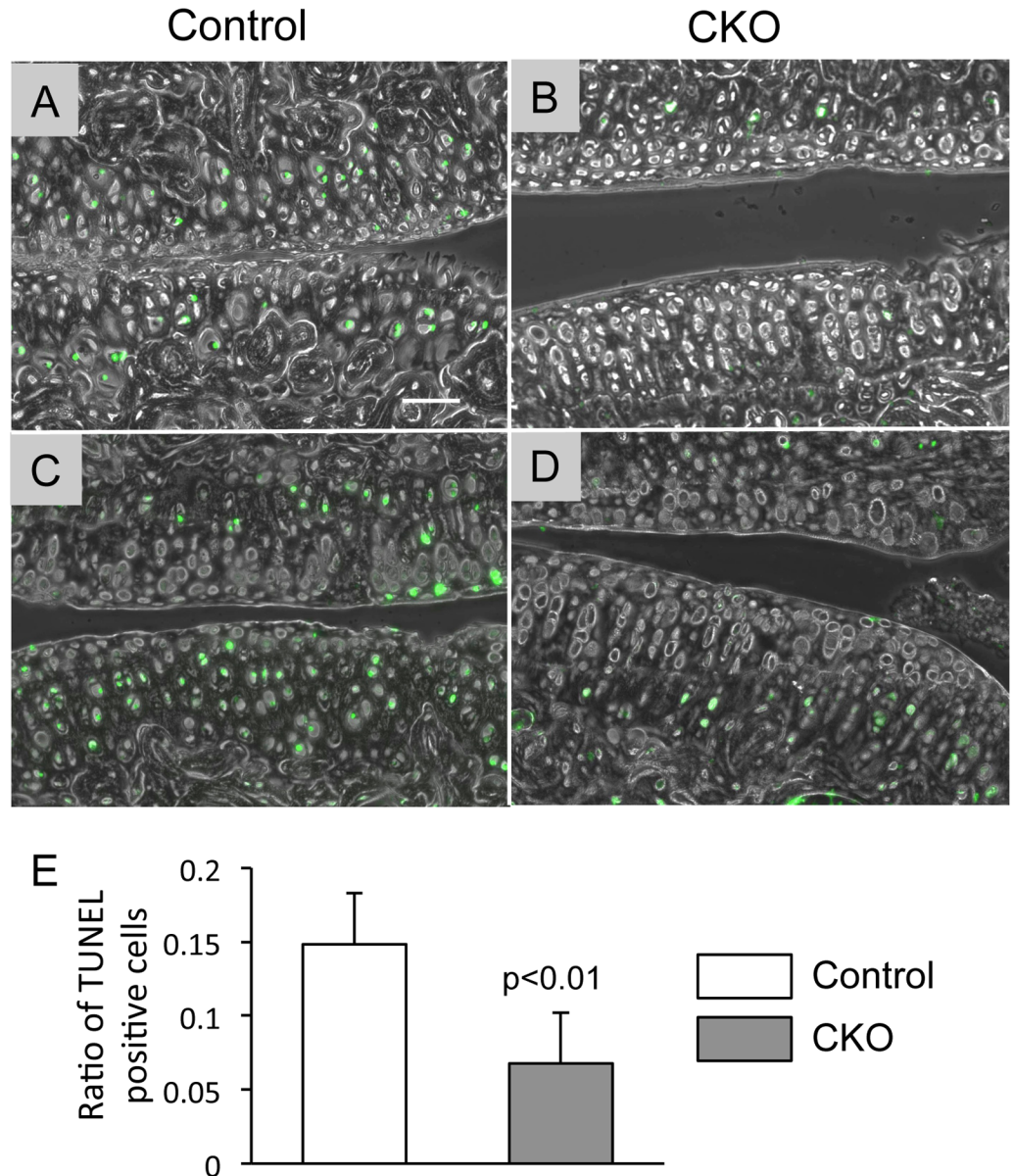


Fig 4. Apoptotic cells in control and $\alpha 5$ CKO articular cartilage after OA surgery. The knee joints were harvested from control (A and C) and $\alpha 5$ CKO (B and D) 4 weeks after OA surgery. The sagittal sections were subjected to TUNEL staining. The fluorescence images (green) were superimposed with the corresponding phase images. Bar, 40 μ m. E, The ratio of the TUNEL-positive cells to total cells was calculated. The graphs represent average and SD (n = 6/group).

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also collagen 10-positive cells throughout the entire zones (S2G and S2H Fig). The MMP13-positive cells were found in both $\alpha 5$ CKO (S2K and S2L Fig, arrows) and control (S2I and S2J Fig, arrows) articular cartilage. The VDIPEN staining revealed that the neo-products of aggrecan cleaved by MMPs were present in both the control and $\alpha 5$ CKO articular cartilage (S2 Fig, VDIPEN, arrows).

3. Analysis of synovial changes in the $\alpha 5$ CKO mice

The knee joints of the control mice showed more evident hyperplasia of synovium (Fig 5A and 5C) compared to the $\alpha 5$ CKO mice (Fig 5B and 5D). The control synovial lining tissue had multi-layer of cells with high cellularity (Fig 5C, double headed arrow) containing CD31-positive vessels (Fig 5E, arrows). The difference in synovial change between the control and the $\alpha 5$ CKO group was confirmed by semi-quantitative analysis (Fig 6). Moreover, the margin of medial tibia in control mice contained ectopic cartilage (Fig 5G), while that of the $\alpha 5$ CKO mice did not (Fig 5H).

4. $\alpha 5$ integrin expression in the OA joints

We examined changes in $\alpha 5$ integrin expression after OA surgery. The staining of $\alpha 5$ integrin was weaker in the OA articular cartilage than that in the normal articular cartilage (Fig 7A vs 7B). In contrast, $\alpha 5$ integrin was found throughout the hyperplastic lesion of the synovium (Fig 7F). These findings suggest that ablation of $\alpha 5$ integrin in the synovium results in reduction in synovial changes. To examine how Gdf5-lineage cells behave in OA, the *Gdf5;Rosa-GFP* reporter mice received OA surgery. Four weeks after surgery we examined which cells express GFP to monitor the distribution of GDF5-lineage cells in the knee joints. As previously reported, the entire articular cartilage is composed of GFP-positive cells (S3 Fig). Interestingly, GFP expression was weaker at the surgery site than at the contralateral site (S3 Fig), suggesting that the basal level of protein expression may decrease in the OA articular cartilage. GFP expression was found in the synovial cells that represent hyperplasia (S3 Fig) at the surgery site, indicating the synovial changes involve Gdf5-lineage cells.

Discussion

Results in this study demonstrated that while $\alpha 5$ integrin may not be essential for synovial joint development and structural maturation, it can play an important role in osteoarthritic changes in articular cartilage and synovial tissue. Osteoarthritis is the most common joint disease and causes clinical symptoms, including pain and stiffness. The results from this study provide important insights into the mechanism of osteoarthritis and would pave the way toward the development of new and effective therapeutic drugs to prevent or ameliorate trauma-induced OA.

The role of $\alpha 5$ integrin in chondrocytes

We observed here that ablation of $\alpha 5$ integrin reduced matrix loss and structural changes in articular cartilage after OA surgery. The $\alpha 5$ CKO articular cartilage showed smaller values of the modified Mankin score and OARSI score (Fig 2H and 2G). Furthermore, the $\alpha 5$ CKO articular cartilage also had a higher nanoindentation modulus (Fig 3), indicating a better load bearing function than wild-type cartilage. At the cellular level, the number of apoptotic cells was significantly smaller in the $\alpha 5$ CKO than that in the control (Fig 4). The finding suggests that $\alpha 5$ integrin may regulate osteoarthritic responses through mediating the apoptotic signal. Pulai et al. [14] have demonstrated that incubation with the $\alpha 5\beta 1$ antibodies induces cell death of articular chondrocytes in suspension culture and concluded that $\alpha 5\beta 1$ integrin provides matrix survival signals. In contrast, we observed a decrease in apoptotic cells in the $\alpha 5$ CKO articular cartilage, suggesting that $\alpha 5$ integrin participates in cell apoptosis. The discrepancy can be accounted for through the following explanations. First, previous findings are based on *in vitro* culture experiments while the results in this study were from *in vivo* experiments. Articular chondrocytes behave differently *in vitro* than *in vivo*. Furthermore, the articular chondrocytes

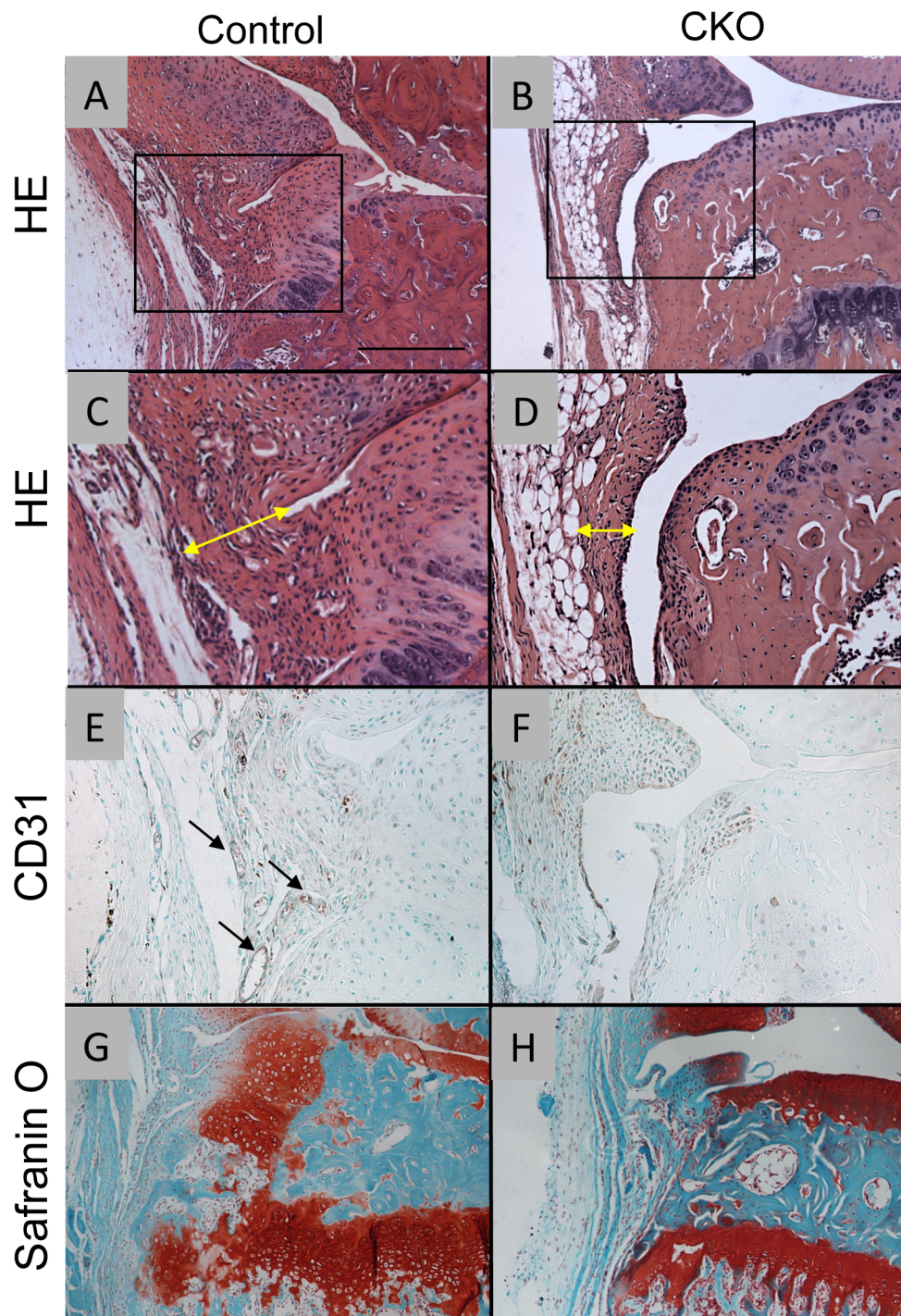


Fig 5. Synovial changes in control and $\alpha 5$ CKO mice after OA surgery. The knee joints were harvested from control (A, C, E and G) and $\alpha 5$ CKO (B, D, F and H) 4 weeks after OA surgery. The sagittal sections were subjected to hematoxylin-eosin staining (HE, A-D), CD31 immunostaining (E and F) and Safranin O staining (G and H). C and D are magnified images of the box of A and B, respectively. Double headed arrows indicate thickening of synovium (C and D). Arrows indicate CD31-positive vessels (E). Bar, 250 μ m for A, B, G and H; 125 μ m for C-F.

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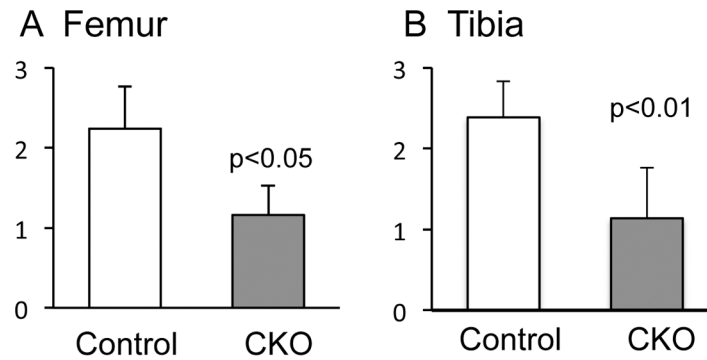


Fig 6. Scores of synovial changes in control and $\alpha 5$ CKO mice after OA surgery. Synovial changes at the medial sites beside the femur (A) and the tibia (B) were evaluated in the control and $\alpha 5$ CKO mice 4 weeks after OA surgery. The graphs represent average and SD, $n = 9/\text{group}$.

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are influenced *in vivo* by many factors that are locally provided in other joint tissues and systemically circulated. Second, most previous studies have used functional antibodies against $\alpha 5\beta 1$ integrin to inactivate $\alpha 5\beta 1$ integrin while our study involves ablation of $\alpha 5$ integrin alone. Functional antibodies might induce other confounding intracellular signaling in addition to the deletion of $\alpha 5$ -mediated signal.

Previous reports have demonstrated that $\alpha 5\beta 1$ integrin mediates matrix degradation induced by fibronectin fragments in cultured chondrocytes [15]. Furthermore, the expression of inflammatory-related molecules, IL-1 β , IL-6, IL-8, PGE2 and nitrite (NO) were stimulated in articular chondrocytes when they are treated with the functional antibodies of $\alpha 5\beta 1$ [17]. The phenotype of the $\alpha 5$ CKO mice observed in this study is likely consistent with these findings. The levels of fibronectin [26, 27] and fibronectin fragments (30–200 kD) are increased in OA articular cartilage and synovial fluid [28]. Fibronectin fragments are strong stimulators of MMPs [29]. Therefore, the absence of $\alpha 5$ integrin may attenuate fibronectin fragment-induced matrix degradation in the $\alpha 5$ CKO mice. It has been demonstrated that $\alpha 5$ integrin expression increased in articular cartilage after partial meniscectomy OA surgery in rats [30, 31] while our results showed reduction in its expression (Fig 7). A decrease in $\alpha 5$ integrin staining was observed particularly in the region that showed severe structural defects. Thus the reduction in $\alpha 5$ integrin expression seen in our model may be associated with reduction in cell metabolic activity or cell death. This assumption is consistent with our findings that articular cartilage at the surgery side contained many apoptotic cells (Fig 4) and a lower level of GFP expression in the *GDF5;Rosa-GFP* mice compared to the articular cartilage at the contralateral side (S3 Fig).

The role of $\alpha 5$ integrin in synovial changes

The OA model we used in this study is a hemisection of medial meniscus in addition to incision of medial collateral ligament, which is a more severe condition than the DMM model that involves transection of the medial meniscotibial ligament [32]. We observed obvious synovial changes at an earlier time point than in the DMM model. The synovium and fat pad expressed $\alpha 5$ integrin at a higher level than articular cartilage. The *Gdf5Cre* system induces Cre activity in articular cartilage but also does so in other joint components, including synovium and fat pad [33]. We demonstrated that thickened synovial tissue is composed of the cells labeled with *Gdf5Cre* (S3 Fig) and showed strong staining for $\alpha 5$ integrin. Thus, deletion of $\alpha 5$ integrin may result in a marked impact on synovium. Indeed, synovial changes in the control mice were much more evident than in the $\alpha 5$ CKO mice.

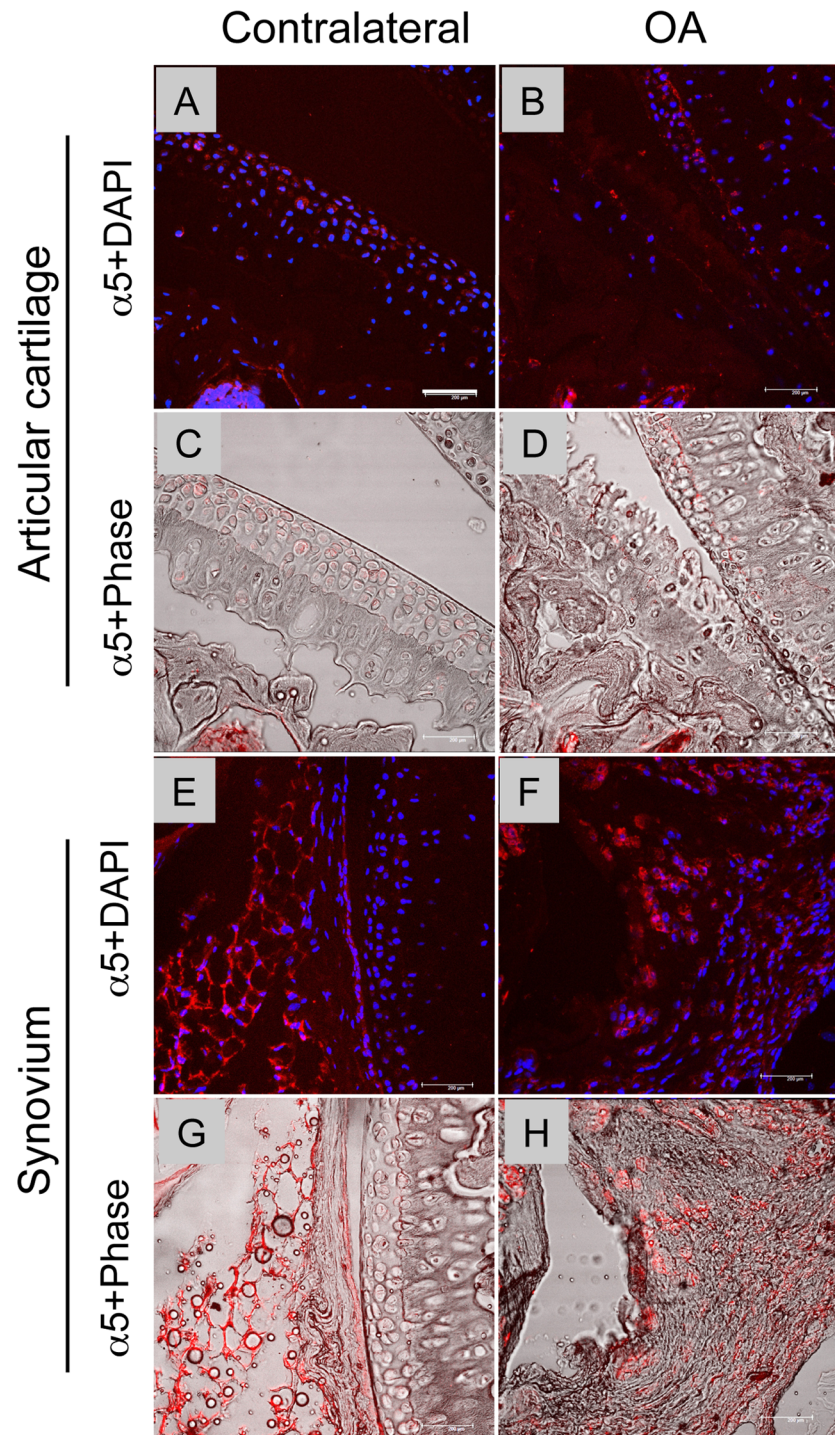


Fig 7. Distribution of $\alpha 5$ integrin in the OA and healthy knee joints. The knee joints were harvested from the surgery site (B, D, F and H) and the contralateral site (A, C, E and G) 4 weeks after OA surgery. A, B, E and F, Superimposed images of $\alpha 5$ integrin staining (red) with DAPI nuclear staining (blue). C, D, G and H, Superimposed images of $\alpha 5$ integrin staining (red) with the phase contrast images. Bar, 40 μ m.

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Synovial changes observed in the control group include hyperplasia of synovium (Fig 5A and 5C), an increase in vessels (Fig 5E), and appearance of ectopic cartilaginous lesions (Fig 5G). In

the $\alpha 5$ CKO group, thickening of the synovium (Fig 5B and 5D and Fig 6) and ectopic cartilage formation (Fig 5H) were to a lesser extent, indicating that $\alpha 5$ integrin mediates these changes directly or indirectly. $\alpha 5$ integrin has been demonstrated to regulate cell proliferation via cell adhesion or spreading [34, 35]. Furthermore, $\alpha 5$ and αv integrins contribute to embryonic vascular development and tumor angiogenesis [9, 36]. Thus, contribution of $\alpha 5$ integrin to hyperplasia of the synovium and vessel formation may be direct. Previous reports have shown that $\alpha 5$ integrin is involved in up-regulation of MMPs (MMP-1, 3 and 13) and inflammatory cytokines (IL-1 α and IL-1 β) in synovial cells in response to fibronectin fragments [37, 38], suggesting that $\alpha 5$ integrin-mediated up-regulation of MMPs and inflammatory cytokines in synovial cells may result in stimulation of loss of matrix and destruction of cartilage. Requirement of $\alpha 5$ integrin has been reported in growth plate chondrocytes in suspension culture [39]. Therefore, absence of $\alpha 5$ integrin would inhibit the growth of ectopic cartilaginous lesions. We need further investigation to elucidate how $\alpha 5$ integrin participates in induction of ectopic cartilage formation.

Conclusions

In summation, our results demonstrate that ablation of $\alpha 5$ integrin in synovial joints reduces susceptibility to osteoarthritic changes in mice and suggest that $\alpha 5$ integrin is a stimulator of osteoarthritis.

Supporting Information

S1 Fig. Expression of $\alpha 5$ integrin in synovial joints in control and $\alpha 5$ CKO mice. The knee joints were harvested at P0 (A and B) or 4 weeks of age (C-F) from the C57BL/6j mice (A and B), control (C and D) and the $\alpha 5$ CKO (E and F) mice. The $\alpha 5$ staining images (red) are superimposed with DAPI nuclear staining (blue). B is the magnified image of the box in A. Bar, 40 μ m. (TIF)

S2 Fig. Apoptotic cells, expression of collagen 10 and MMP13 and cleaved aggrecan products in control and $\alpha 5$ CKO articular cartilage after OA surgery. The knee joints were harvested from control (A, B, E, F, I, J, M and N) and $\alpha 5$ CKO (C, D, G, H, K, L, O and P) 4 weeks after OA surgery. The sagittal sections were subjected to TUNEL (A-D), collagen 10 (E-H), MMP13 (I-L) and VDIPEN (M-P) staining. B, D, F, H, J, L, N and P are magnified images of the box of A, C, E, G, I, K, M and O, respectively. Arrows indicate the cells or regions that represent positive immuno-reactivity to the corresponding staining. Bar, 250 μ m for A, C, E, G, I, K, M and O; 125 μ m for B, D, F, H, J, L, N and P. (TIF)

S3 Fig. Expression of GFP in the knee joints after OA surgery. Sagittal sections were made from the OA surgery knee joint (A and C) and the contralateral knee joint (B and D) in the Gdf5Cre;Rosa-GFP mouse and subjected to GFP immunostaining. A and B, articular cartilage. C and D, synovium adjacent to medial tibia. Bar, 125 μ m. (TIF)

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Author Contributions

Conceived and designed the experiments: MEC LH MEI. Performed the experiments: MEC CW ATG KZ LC RY YU NF MEI. Analyzed the data: MEC CW KZ YU MI AF YZ LQ LH

MEI. Contributed reagents/materials/analysis tools: AF LH. Wrote the paper: MEC LC LH MEI.

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