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**Supplementary materials and methods**

**Mice**

Col2-CreERT and c-fos\textsuperscript{flx/flx} mice and alleles (MGI:2679338, MGI:3665440) were previously described. Col2-CreERT and c-fos\textsuperscript{flx/flx} mice were maintained on pure C57BL/6J background. Male mice were used for the all experiments. Randomized block design was used to organize the experimental cohorts. Mice were housed in Specific Pathogen-Free environment with free access to food and drink. All animal experiments were conducted according to institutional policies and national and European guidelines. Male mice were injected with tamoxifen (TAM) intraperitoneally at two time points (at 2.5 weeks and 9 weeks of age, 2 mg/mouse/day, five consecutive days). Mice were subjected to DMM or sham surgery at 10 weeks of age. In brief, the joint capsule was opened immediately after anaesthesia, and the medial meniscotibial ligament was cut to destabilize the meniscus without damaging other tissues. In sham surgery, the joint capsule was opened in the same way but without cutting meniscotibial ligament. The knee joints were collected 8 weeks after the surgery and articular cartilage damages were evaluated by OARSI scoring system\textsuperscript{2}.

**Clinical specimens**

Human articular cartilage was obtained from OA patients during total knee arthroplasty with written informed consent and following the terms of the ethics committee of the Medical University of Vienna (EK-Nr.: 1822/2017, 2166/2020). Cartilage specimens from 20 OA patients (11 females, 9 males; age: 66.8±9.1, age range: 54–83) were histologically processed and stained with safranin O to enable grading of cartilage degeneration according to the Mankin score (MS)\textsuperscript{3}. From each patient, one tissue region with MS≤5 and one region with MS≥8 were selected. Tissue sections were then processed for immunohistochemical staining using an anti-c-Fos antibody (Santa Cruz Biotechnology, Inc., sc-52) and the percentage of immunopositive chondrocytes was assessed in each region\textsuperscript{4}.

**Dichloroacetic Acid treatment**
One week after DMM (11-weeks-old), mice were randomized and treated with DCA (3mg/kg body weight) with drinking water for 7 weeks. The knee joints were collected and articular cartilage damages were evaluated by the OARSI scoring system.

**Immunohistochemistry**

Knee joints were dissected, and decalcified with 18% EDTA (pH 8.0) for two weeks before paraffin embedding for histology, including, safranin O/fast green staining, picrosirius red staining and/or IHC. Immunohistochemistry/immunofluorescence was performed on 5 µm thick sections. Slides were deparaffinised using xylene or citrus reagents and bathed in decreasing alcohol concentrations (100%-96%-70%-ethanol) followed by water washes. Deparaffinised tissue sections were treated with H₂O₂ for 30 min for IHC and antigen retrieval was carried out using citrate buffer and a pressure cooker for 20 min. After permeabilisation by 0.1% TritonX-100/PBS for 10 min, non-specific binding was blocked with 10% normal serum/PBS. Tissue sections were incubated with primary antibodies or isotype controls (Supplemental Table 2) overnight at 4°C. Biotin/streptavidin amplification and HRP-based chromogen detection (VECTASTAIN ABC Kit or M.O.M.® detection Kit, Vector Laboratories, Inc) were used for IHC following the manufacturer’s instructions, while IF sections were incubated with Alexa Fluor® 488 or 555 goat anti-rabbit IgG (H+L) for one hour. Positive area/cells within the articular cartilage region were quantified using ImageJ. Representative IHC/IF images for Isotype control stainings are show in Suppl. Figure 9.

**Safranin O/fast green staining**

Dепaraffinised tissue sections were stained with Fast Green FCF (Sigma) for 15 min at room temperature. After washing with 1% acetic acid solution for 10 -15 sec, samples were stained with Safranin O (Sigma) for one hour and samples were dehydrated in absolute alcohol, incubated in xylene and mounted using permanent non-aqueous mounting media (Entellan® new, Sigma-Aldrich). Briefly, 2 - 4 sections within every consecutive 6 - 7 regions in the entire articular cartilage for each knee joint were stained with Safranin O/Fast green. The OARSI scoring system was used to assess degenerative changes resulting from the surgery by two independent, blinded reviewers. The medial and lateral side of knee received a total severity
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score resulting from the seven (Figure 2D and Supplementary figure 2D) or ten (Figure 6C) highest section scores depending on the experiment. Articular cartilage and glycosaminoglycan (GAG) positive areas in the image were measured by ImageJ. Safranin O stained areas were quantified as GAGs areas and relative GAGs (%) areas were calculated by the formula: (GAG area)/(articular cartilage area) x 100. The degree of synovitis and osteophyte formation were evaluated as previously reported, respectively\(^5, 6\). To measure chondrocyte number, randomly selected several sections from each knee stained with Safranin O/Fast green were quantified by ImageJ. The final measurement is an average of these sections.

**Picrosirius red staining**

Deparaffinised tissue sections were stained with Direct Red 80 in Picrosirius acid (Sigma) for one hour at room temperature. After two washes in 0.5% acetic acid in distilled water, samples were dehydrated in absolute alcohol, incubated in xylene and mounted using permanent non-aqueous mounting media. Images were acquired with a motorized microscope (BX63, Olympus) using both bright-field and polarized light (2 - 3 images/knee joint). Articular cartilage regions were extracted from the polarized light images and collagen area positivity was quantified and compared with the total cartilage area by ImageJ. Fibrillar collagen parameters were quantified by the open source tool: CurveAlign 4.0 framework (LOCI, University of Wisconsin System). Horizontally set polarized light images were converted into grayscale and individual collagen fiber length was extracted and computed by CT-FIRE.

**RNA isolation and qPCR**

Total RNA was isolated using TRI reagent (Sigma-Aldrich), and complementary DNA was synthesized using Ready-To-Go-You-Prime-First-Strand Beads (GE Healthcare) or GoScript™ Reverse Transcription Mix, Oligo(dT) (Promega) and qPCR used GoTaq qPCR Master Mix (Promega) and Eppendorf fluorescence thermocyclers, all according to the manufacturer’s instructions. The \(2^{\Delta\Delta CT}\) method was used to quantify the amplified fragments. Expression levels were normalized using at least one housekeeping gene. Primer sequences are listed in Supplemental Table 1.
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**Bulk RNA-sequencing**

For bulk RNA-sequencing, mice were sacrificed 4 weeks after DMM and articular cartilage from DMM-treated and contralateral sides of knee joints was carefully dissected by a surgery scalpel using binocular and immediately processed for RNA isolation by TRI Reagent. Sequencing libraries were prepared using the NEBNext Ultra II RNA Library Prep and sequenced on an Illumina NextSeq500 platform in a 75-base pair single-read mode. Sequencing data were then mapped to the Mus musculus/mmc10 assembly of the mouse genome with the Spliced Transcripts Alignment to a Reference (STAR) aligner, and differential gene expression was analysed using DESeq2 with Benjamini-Hochberg adjusted p-values. Differentially expressed genes were considered statistically significant with an adjusted P < 0.05. RNA-seq datasets are deposited in the Gene expression omnibus archive https://www.ncbi.nlm.nih.gov/geo/ as series: GSE228469 available at the time of publication.

**Ingenuity Pathway Analysis**

We used the “Upstream Regulator Analysis” tool implemented in the Ingenuity Pathway Analysis suite (IPA, Ingenuity Systems, Inc., Redwood City, CA, USA) to identify upstream regulators that may be responsible for the gene expression changes in the dataset. This analysis seeks to identify upstream regulators and predict whether they are activated or inhibited given the observed expression changes of their downstream targets, without taking into account expression of the upstream regulators themselves. We focused our analysis on transcription regulators as well as secreted proteins for which an activation state prediction could be generated, and ranked them based on the p-value generated by IPA for their overlap with the expected causal effects on their targets. Genes with an overlap p value < 0.05 (Fisher's exact test) by IPA were predicted to be upstream regulators as described previously.

**Gene set enrichment analysis**

Biological insights concerning the differentially expressed genes were explored via gene set enrichment analysis (GSEA). The analysis was performed with the GSEA software and gene sets downloaded from the Molecular Signature Database (www.gsea-msigdb.org/gsea/msigdb/). In addition, one custom gene set was used from RNA-seq data of
human primary articular chondrocytes comparing OA patients to healthy individuals (E-MTAB-2723). P-values and the false discovery rate (FDR) for the enrichment score of each gene set were calculated based on 1000 gene set permutations and statistical significance (nominal P value) of the Enrichment Score calculated using an empirical phenotype-based permutation test.\(^{34}\)

\textit{In situ pyruvate dehydrogenase activity assay}

We adapted the colorimetric enzyme histochemistry assay for PDH and LDH activity assay\(^ {42-45}\). Mice were sacrificed 4 weeks post-DMM and knee joints from DMM and contralateral sides were dissected and immersed with OCT compound (Sakura Finetek, Tokyo, Japan). Frozen sections of 16 \(\mu\)m thickness were prepared for the assay and sections were placed onto the glass slides which were kept at -80°C until further usage. The tissue sections were incubated with the mastermix reagent with/without inhibitor (50 \(\mu\)l/tissue section). For \textit{in situ} PDH activity assay, the mastermix was prepared as follows: pyruvate (final concentration 340 mM), coenzymes (final concentration 16 mM TPP, 10 mM CoA, 7 mM NAD, 10 mM FAD), sodium azide (final concentration 5 mM), methoxyphenanzine methosulfate (final concentration 0.45 mM). The pre-warm (60 °C) NBT (final concentration 5 mM) and tris-maleate buffer (final concentration 120 mM, pH8.0) were added to the mastermix, vortexed rigorously and centrifuged at 14,000 \(\times\) g for 2 min. Tissues were incubated with supernatants from the reaction mastermix for 30 min at 37 °C. For \textit{in situ} LDH activity assay, the mastermix was prepared as follows: lactate (final concentration 15 mM), sodium oxamate (final concentration 100 mM for LDH inhibitor reaction), coenzyme (final concentration 3 mM NAD), sodium azide (final concentration 5 mM), methoxyphenanzine methosulfate (final concentration 0.45 mM). The pre-warm NBT (final concentration 5 mM) and tris-maleate buffer (final concentration 100 mM, pH7.5) were added to the mastermix. Tissues were incubated with the mastermix for 7 min at room temperature. The reaction was stopped by washing out the mastermix with warm PBS (60 °C) and nuclei were stained with DAPI. RGB Images were acquired with a motorized microscope (BX63, Olympus) using bright-field. The RGB image with pink/violet color (formazan products) was converted to a monochrome image and then inverted by Photoshop.
CS6 for signal intensity measurements. The value of the color intensity of the image from the unstained cartilage section (no reaction mastermix and no DAPI) was used as a background intensity value and it was subtracted from the value of the color intensity of the stained sample. The total cell number was calculated with DAPI. Quantification of color intensity value was performed using ImageJ. Integrated density (IntDel) in cartilage was calculated and the values were divided by total cells (IntDel/cell).

**Cell culture**

Primary mouse chondrocytes were isolated and cultured from the knee joint of P5 c-Fos\(^{WT}\) mice\(^{10}\). Cells were plated at 5×10\(^5\) cells in 100 cm\(^2\) dishes and transfected with adenovirus (Ad5CMVCre/empty, University of Iowa healthcare, Viral Vector Core Facility) at 100 MOI and cultured for 48 hours. After removing the media, cells were cultured for 48 hours with/without 10mM DCA and processed to RNA extraction and/or in vitro Pdh activity assay. For in vitro Pdh activity assay, cells were harvested and Pdh activity was measured by the Pdh activity assay kit (Sigma) with a 1420 Multilabel Counter Victor 3 (Perkin Elmer).

**Statistical analysis**

Methods for statistical evaluation of RNA-seq data are indicated in each respective chapter above. For the rest of the data, Prism8 (GraphPad) was used for analyses and statistics. Statistical significance was determined using paired or unpaired t-test (two-tailed) or Mann-Witney test according to sample distribution for comparing two groups of samples. Two-way ANOVA with Bonferroni post-test was applied for grouped or multivariate analysis, as appropriate. If the variances were significantly different, as measured by the f-test, an unpaired two-tailed Student's t-test with Welch's correction was applied. Unless otherwise specified, data are shown as mean ± s.d., and a p-value below 0.05 is considered statistically significant and indicated as *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001.
Supplementary references


Supplemental Figure 1. Matsuoka K. et al.

A

Sham | DMM

2 weeks

Sham (n = 5) | DMM (n = 5)

8 weeks

Sham (n = 10) | DMM (n = 11)

B

% p-c-Fos positive cells

Sham (n = 7) | DMM (n = 10)

2 weeks

Sham (n = 9) | DMM (n = 16)

8 weeks

C

Sham | DMM

c-Jun

8 weeks

Sham (n = 6) | DMM (n = 9)

D

% p-c-Jun positive cells

Sham (n = 10) | DMM (n = 11)

E

Sox9

Sham | DMM

F

% Sox9 positive cells

Sham (n = 5) | DMM (n = 5)
Supplemental Figure 2. Matsuoka K. et al.

A) 

B) 

C) 

D) 

E) 

F) 

G) 

H) 

I) 

Supplemental material
Supplemental Figure 3. Matsuoka K. et al.

A

B

C

D

E

F

G

Supplemental material

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2023; Ann Rheum Dis, et al. Matsuoka K
Supplemental Figure 4. Matsuoka K. et al.
Supplemental Figure 5. Matsuoka K. et al.

A) Glycolysis

B) ldha

C) pdk1

D) c-fos

E) Egln3

F) Psmb9

Supplemental Figure 5. Matsuoka K. et al.
Supplemental Figure 6. Matsuoka K. et al.
Supplemental Figure 7. Matsuoka K. et al.

A

Pyruvate (+)

DCA

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- DMM -

B

Enzyme activity (IntDel/cell)

DCA

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**P = 0.047

C

Lactate (+)

DCA

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- DMM -

D

Enzyme activity (IntDen/total cell)

DCA

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**P = 0.0058

* P = 0.039

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Supplemental Figure 8. Matsuoka K. et al.
Supplemental Figure 9. Matsuoka K. et al.
Supplemental Figure legends

Supplemental Figure 1. Expression of phosphorylated c-Fos and c-Jun/phosphorylated c-Jun in healthy and OA articular chondrocytes

(A) Representative IHC images of phosphorylated c-Fos (p-c-Fos) at 2 and 8 weeks post-surgery. (B) Quantification of p-c-Fos positive cells. Red arrows indicate positive cells. (C) IHC images of c-Jun and p-c-Jun at 8 weeks post-surgery (D) Quantification of c-Jun and p-c-Jun positive cells. (E) IHC images of Sox9 at 8 weeks post-surgery. (F) Quantification of Sox9-positive cells. Bar graphs and plots represent or include mean ± s.d. *P < 0.05, **P < 0.01, and ***P < 0.001. Statistical differences between groups were analysed by two-way ANOVA with Bonferroni post hoc analysis in B and by non-parametric Mann-Whitney test in D and F.

Supplemental Figure 2. Characterization of knee joints from c-FosWT mice and c-FosCh mice at 8 weeks after the surgery.

(A) Experimental procedure to assess the long-term effect of c-fos deletion in chondrocytes. Tamoxifen was injected to mice at two time points (2.5 and 9 weeks of age, 2mg/mouse/day, 5 consecutive days) and mice are kept for 1 year. (B) Representative images of safranin O/fast green staining and GFP positive articular chondrocytes of knee joints from one-year-old c-FosWT mice and c-FosCh mice. (C) Relative cartilage area quantified by ImageJ analysis. (D) Relative glycosaminoglycan area quantified by ImageJ analysis. Bar graphs and plots represent or include mean ± sd, respectively. (E) Representative images of safranin O/fast green staining of knee joints from c-FosWT mice and c-FosCh mice at 2 and 8 weeks post-surgery. Osteophyte in the DMM-treated group: Quantification of osteophyte maturity (F) and area (G). (H) Representative images of the synovial membranes 2 weeks post-DMM. (I) Synovial inflammation score in DMM-treated groups. Bar graphs and plots represent or include mean ± sd, respectively. *P < 0.05, **P < 0.01 and ***P < 0.001. Statistical differences between groups were analysed by non-parametric Mann-Whitney test (C, D) and by two-way ANOVA with Bonferroni post hoc analysis (F, G and I).

Supplemental Figure 3. Characterization of anabolic/catabolic factors in articular
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cartilage from c-Fos<sup>WT</sup> mice and c-Fos<sup>Ch</sup> mice 8 weeks post-surgery.

(A) Representative images of PCNA and (B) quantification of positive cells. Positive cells are indicated by red arrows. (C) Representative images of picrosirius red staining of knee joints. Pictures are taken under the optical and polarized light and the polarized image is converted to a Black and White image for fiber analysis. Individual fiber is extracted from multiple images by CurveAlign and the mean value of fiber length is calculated. (D) Representative images of MMP-13 and (E) quantification of positive cells. Positive cells/area are indicated by white arrows. (F) Representative images of Adamts5 and (G) quantification of positive cells. Positive cells are indicated by red arrows. Bar graphs and plots represent or include mean ± s.d., respectively. *P < 0.05 and **P < 0.01. Statistical differences between groups were analysed by two-way ANOVA with Bonferroni post hoc analysis.

Supplemental Figure 4. GSEA analysis and enriched pathways.

(A) Quantification of cartilage damage 4 weeks post-surgery. OARSI scores from sham-treated c-Fos<sup>WT</sup> and c-Fos<sup>Ch</sup> mice were similar and therefore pooled in a single bar. Statistical differences between groups were analysed by non-parametric Mann-Whitney test. (B) Bulk RNA-sequencing of articular cartilage from DMM-treated mice. Venn diagram showing DEGs overlaps between two datasets. The Indicated number of genes are unique to each data-set. Dataset 1 (172 genes, yellow): contralateral side (n = 4) vs DMM-treated side from c-Fos<sup>WT</sup> mice (n =3). Dataset 2 (4394 genes, violet): c-Fos<sup>WT</sup> mice (n =3) vs c-Fos<sup>Ch</sup> mice (n = 4) in the DMM-treated side. The number (628) in the overlapping area indicates shared gene between two datasets. (C) Heatmap of DEGs (725 genes) shared between the two datasets. Red and green correspond to genes with statistically significant up- and downregulation, respectively (P-value < 0.05). (D) Log2FC-based relative mRNA expression heatmap of top-ranked factors enriched in biological groups shown in Figure 4C and Supplemental Figure 4A. Asterisk indicates P < 0.05 (black), P < 0.01 (blue) and P < 0.001 (red). (E) IPA-predicted upstream secreted proteins from c-Fos<sup>Ch</sup> mice over c-Fos<sup>WT</sup> mice, showing activation Z-score (bars) and Log2FC (asterisk indicates P-value < 0.05). (F) Relative mRNA expression of IPA-predicted TFs from DMM-treated side over contralateral side in c-Fos<sup>WT</sup> mice. Bar graphs and
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plots represent or include mean ± s.d., respectively. *P < 0.05, **P < 0.01, and ***P < 0.001. Statistical evaluation of RNA-seq data was performed as indicated in methods.

Supplemental Figure 5. c-Fos deficiency increases Hif-1α and Pdk1 expression in experimental OA

(A) Relative mRNA expression heatmap of factors in glycolysis, PDH-PDK pathway and TCA cycle based on Log2FC in the DMM-treated side over contralateral side in c-FosWT mice. Asterisk indicates *P < 0.05 (black), *P < 0.01 (blue) and *P < 0.001 (red). Statistical evaluation of RNA-seq data was performed as indicated in methods and gene-set enrichment P values < 0.05 and FDR q<0.25 was deemed significant. (B) qPCR analysis of ldha, (C) pdk1, pdha, pdhx and dld gene in articular cartilage of contralateral and DMM-treated site from c-FosWT mice and c-FosChm mice. (D) qPCR analysis of primary articular chondrocytes derived from c-FosWT mice infected with either empty adeno-empty) or Cre-recombinase- (adeno-Cre) expressing Adenoviruses. Gene expression was analysed 48 hours post-Adenovirus infection. (E) Log2FC-based relative mRNA expression heatmap of Hif-1α signaling pathway and proteasome genes. Bar graphs and plots represent or include mean ± s.d., respectively. *P < 0.05. Statistical differences between groups were analysed by two-way ANOVA with Bonferroni post hoc analysis in B and C and by Mann-Whitney test in D.

Supplemental Figure 6. c-Fos deficiency in articular chondrocytes decreases Pdh activity and increases Ldh activity.

(A and B) Representative images of in situ Pdh (A) and Ldh (B) activity assay. The pink/violet colour of bright field images was changed to grey scale. The image was inverted and the total integrated colour intensity in articular cartilage was quantified. In the inverted image, non-specific colour in the bone appears in all tissue sections including the control reaction (non-stained section). (A, right panel) Representative images of in situ enzyme activity assay in the control reactions. (C) Pdh activity assay in primary articular chondrocytes derived from c-FosWT mice treated either adeno-cre or adeno-empty with/without DCA. Bar graphs and plots represent or include mean ± s.d., respectively. *P < 0.05 and ****P < 0.001. In all panels,
statistical differences between groups were analysed by two-way ANOVA with Bonferroni post hoc analysis.

**Supplemental Figure 7. DCA treatment rescues LDH activity in c-Fos deficient mice**

**(A - D)** Mice were subjected with DMM at 10 weeks of age and treated with DCA from at 11 weeks of age for 3 weeks. Knee joints were analysed 4 weeks post-surgery. **(A)** Representative images of in situ Pdh activity assay. **(B)** Quantification of Pdh activity with/without DCA treatment. **(C)** Representative images of *in situ* LDH activity assay. **(D)** Quantification of LDH activity with/without DCA treatment. Bar graphs and plots represent or include mean ± s.d., respectively. *P < 0.05 and **P < 0.01. In all panels, statistical differences between groups were analysed by two-way ANOVA with Bonferroni post hoc analysis.

**Supplemental Figure 8. DCA treatment rescues cell proliferation and collagen synthesis in c-Fos deficient mice**

**(A)** Representative images of Ki67. **(B)** Representative images of picrosirius red staining of knee joints from c-FosWT mice and c-FosΔCh mice treated with or without DCA 8 weeks post-DMM surgery. Pictures are taken under polarized light. **(C)** IF of collagen type 2 (green) of articular cartilage from c-FosWT mice and c-FosΔCh mice with or without DCA 8 weeks post-DMM. Col2 positive area were indicated by white arrows.

**Supplemental Figure 9.** Representative IHC/IF images using isotype rabbit/mouse IgG matching the antibodies used in this study on knee joint sections from sham or DMM-operated c-FosWT mice and c-FosΔCh mice. IHC with **(A)** rabbit IgG and **(B)** mouse IgG, nuclei are counterstained with Hematoxylin. IF with **(C)** rabbit IgG and **(B)** mouse IgG, nuclei are counterstained with DAPI.
Supplemental Table 1. Primers and Plasmids

Oligonucleotide primers for quantitative PCR – mouse genes:

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## Supplemental Table 2. Antibody/Antigen retrieval methods

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| c-Fos    | Santa Cruz Biotechnology (sc-52) | IHC | Mouse tissue 1:200 dilution  
Antigen retrieval: Unitrieve, 60°C for 1 hour  
Human tissue 1:75 Citrate buffer (pH 6.0) in a pressure cooker |
| p-c-Fos  | Home-made Rabbit polyclonal antibody (phospho-Ser 362)(65) | IHC | 1:100 dilution  
Antigen retrieval: Unitrieve, 60°C for 1 hour |
| c-Jun    | Cell signaling technology (60A8) | IHC | 1:100 dilution  
Antigen retrieval: Citrate buffer (pH 6.0) in a microwave |
| p-c-Jun  (Ser73) | Cell signaling technology (D47G9) | IHC | 1:100 dilution  
Antigen retrieval: Citrate buffer (pH 6.0) in a microwave |
| Sox9     | Cell signaling technology (D8G8H) | IHC | 1:100 dilution  
Antigen retrieval: Citrate buffer (pH 6.0) in a microwave |
| Ki-67    | Dako (MIB-1) | IHC | 1:50 dilution  
Antigen retrieval: Citrate buffer (pH 6.0) in a microwave |
| Pcna     | Santa Cruz Biotechnology (sc-56) | IHC | 1:100 dilution  
Antigen retrieval: Citrate buffer (pH 6.0) in a microwave |
| Col2a1   | Santa Cruz Biotechnology (sc-5265) | IHC | 1:100 dilution  
Antigen retrieval: Proteinase K (20μg/mL), 37°C for 20 minutes |
| Mmp-13   | Abcam (AB39012) | IHC | 1:100 dilution  
Antigen retrieval: Proteinase K (20μg/mL), 37°C for 20 minutes |
| Adamts-5 | Abcam (AB41037) | IHC | 1:50 dilution  
Antigen retrieval: trypsin, 37°C for 20 minutes |
| Pdk1     | Santa Cruz Biotechnology (sc-515944) | IHC | Mouse tissue 1:100 dilution  
Antigen retrieval: trypsin, 37°C for 20 minutes  
Human tissue 1:75 |
| Hif-1α   | Novus Biologicals (NB100-105) | IHC | 1:20 dilution  
Antigen retrieval Proteinase K (20μg/mL), 37°C for 25 minutes |
| Normal mouse IgG | Santa Cruz Biotechnology (sc-2025) | IHC | 1:20 dilution  
Antigen retrieval Proteinase K (20μg/mL), 37°C for 25 minutes |
| Normal Rabbit IgG | Sigma Aldrich (12-370) | IHC | 1:100 dilution  
Antigen retrieval: Citrate buffer (pH 6.0) in a microwave |