# Functionalized nanogels with endothelin-1 and bradykinin receptor antagonists peptides decrease inflammatory and cartilage degradation markers of osteoarthritis in a horse organoid model of cartilage

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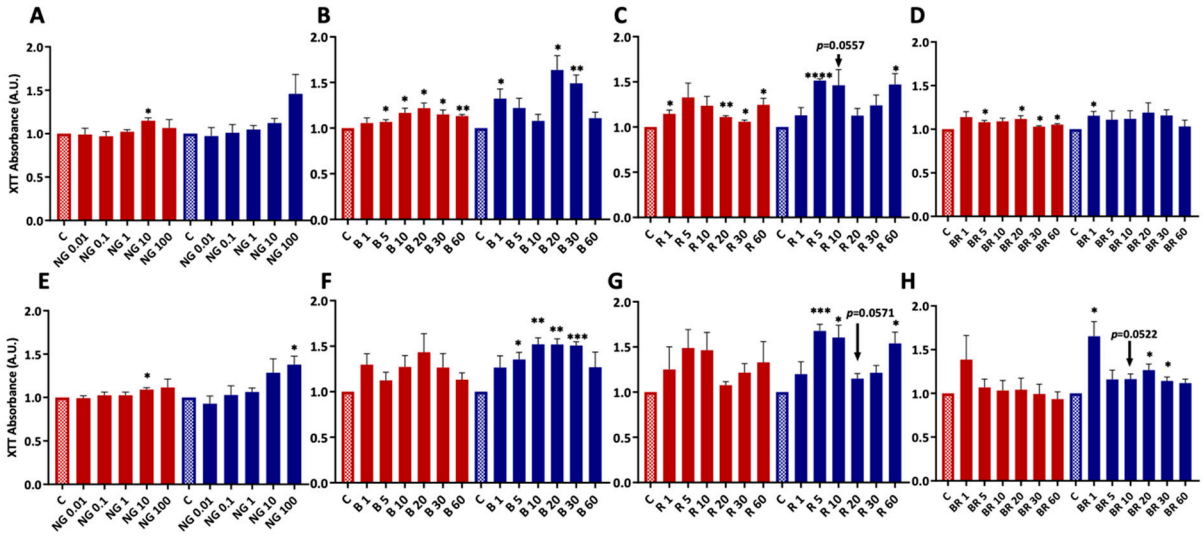


**Figure S1.** Effects of nanogels on the viability and proliferation of equine articular chondrocytes (eACs). eACs were amplified and seeded at the third passage in monolayer (20 000 cells/cm$^2$). At 80% confluence, cells were treated with nanogels formulations, NG at 0.01, 0.1, 1, 10 or 100 µg/mL, NG-BQ-123 (B), NG-R-954 (R) and BR at 1, 5, 10, 20, 30 or 60 nM) and then incubated in normoxia **(red)** or in hypoxia **(blue)** for 24h **(A, B, C, D)** and 48h **(E, F, G, H)**. Control (without nanogels) was included. The levels of formazan were measured in the culture media at the end of each incubation time (XTT kit, Roche). Data are represented as histograms (n=3). Student *t*-tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.001$) were used to compare each treatment with control. Results were normalized to the control. NG: Non-functionalized nanogel, B: BQ-123-CHI, R: R-954-HA, BR: Equimolar combination of BQ-123-CHI and R-954-HA, C: Control, A.U.: Arbitrary Units.

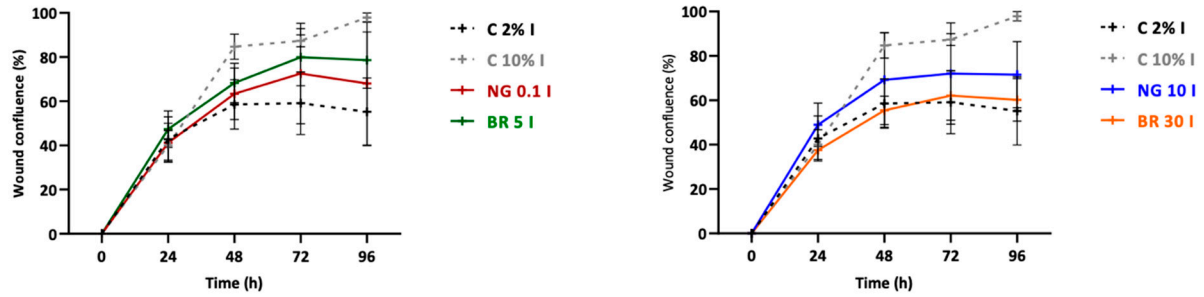


**Figure S2.** Effects of nanogels on wound filling in presence of IL-1β. Equine articular chondrocytes (eACs) were amplified and seeded at the third passage in monolayer (20 000 cells/cm$^2$). At 100% of confluence, a wound filling was assayed in each well and nanogel treatments were added to cells, 0.1 and 10 µg/mL NG, 5 and 30 nM BR (in the presence of 2% FBS (C 2%)), with IL-1β (10 ng/mL) and proliferation was followed by IncuCyte®. The cells were also incubated in the presence of 10% FBS (C 10%). At the end of the incubation period, confluence and wound area were analyzed with ImageJ software. Data are represented as curves (n=3). NG: Non-functionalized nanogel, BR: Equimolar combination of BQ-123-CHI and R-954-HA, C: Control, I: IL-1β.

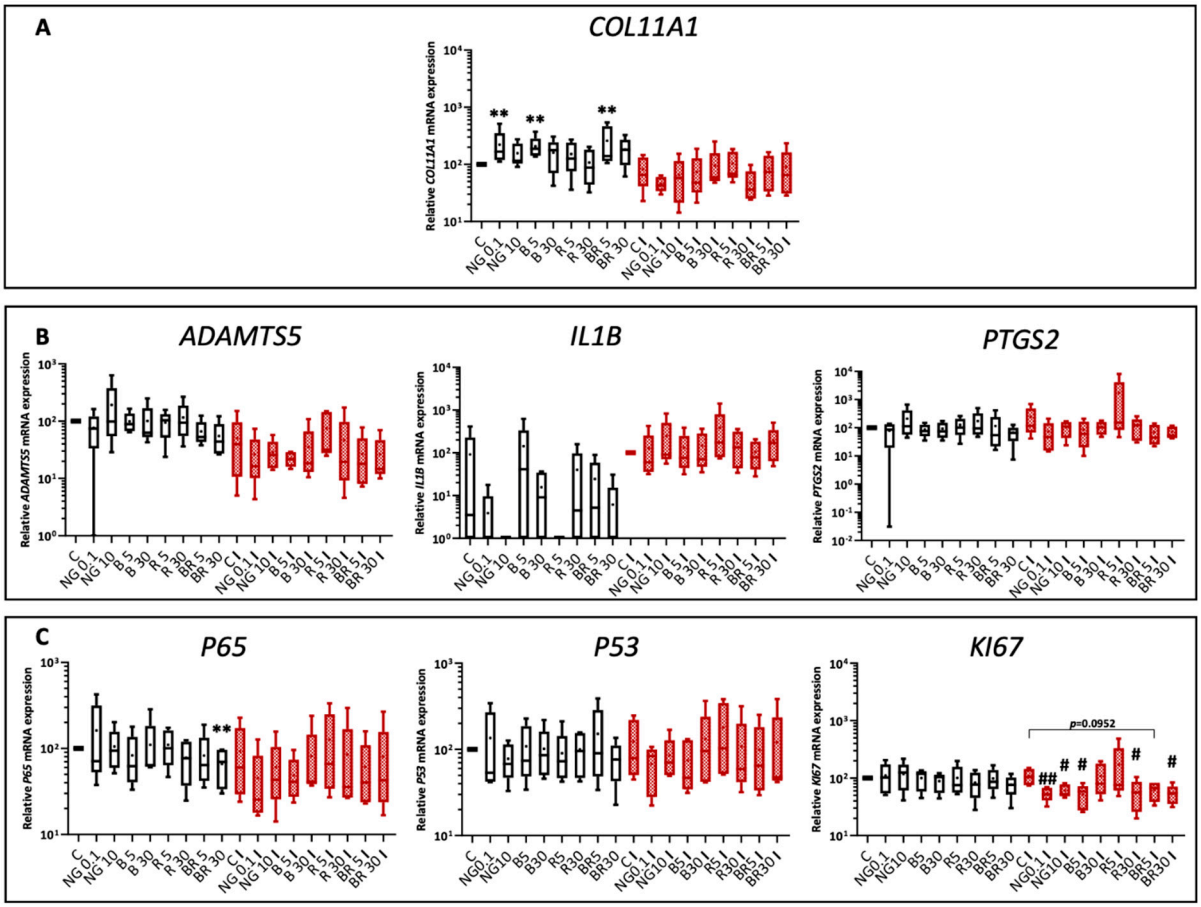


**Figure S3.** Other markers gene expression analysis of equine articular chondrocytes (eACs) cultured with nanogels and IL-1β. eACs were grown in type I/III collagen sponges and then incubated for 7 days in hypoxia in the absence (C) or presence of nanogel formulations (NG at 0.1 and 10 µg/mL, B, R and BR at 5 and 30 nM) and in the absence **(black)** or presence **(red)** of IL-1β (C I) (10 ng/mL). Transcript expression of some matrix **(A)**, catabolic and inflammatory **(B)** and others **(C)** markers are shown in arbitrary units. The results are shown as box plots (n=5). Statistical analyses were performed by using a Mann-Whitney test, significantly (# $p < 0.05$, **, ## $p < 0.01$) different from C (*) and C I (#). NG: Non-functionalized nanogel, B: BQ-123-CHI, R: R-954-HA, BR: Equimolar combination of BQ-123-CHI and R-954-HA, C: Control, I: IL-1β.

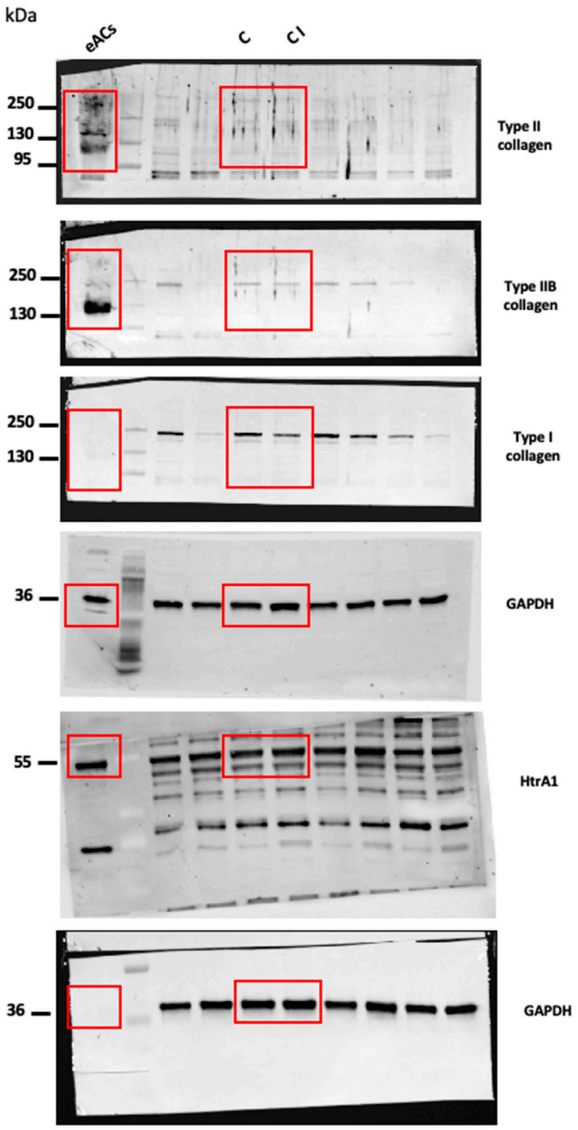


**Figure S4.** Complete gel and PVDF membranes analyzed in the western blots. For the western blots presented in Figure 9A, the entire images of the membranes captured with the Chemidoc MP Imaging System (Bio-Rad) are shown. The molecular weight marker (MW, kDa) is indicated on the left side of the images. Red boxes indicate the cropped images shown in Figure 9A.

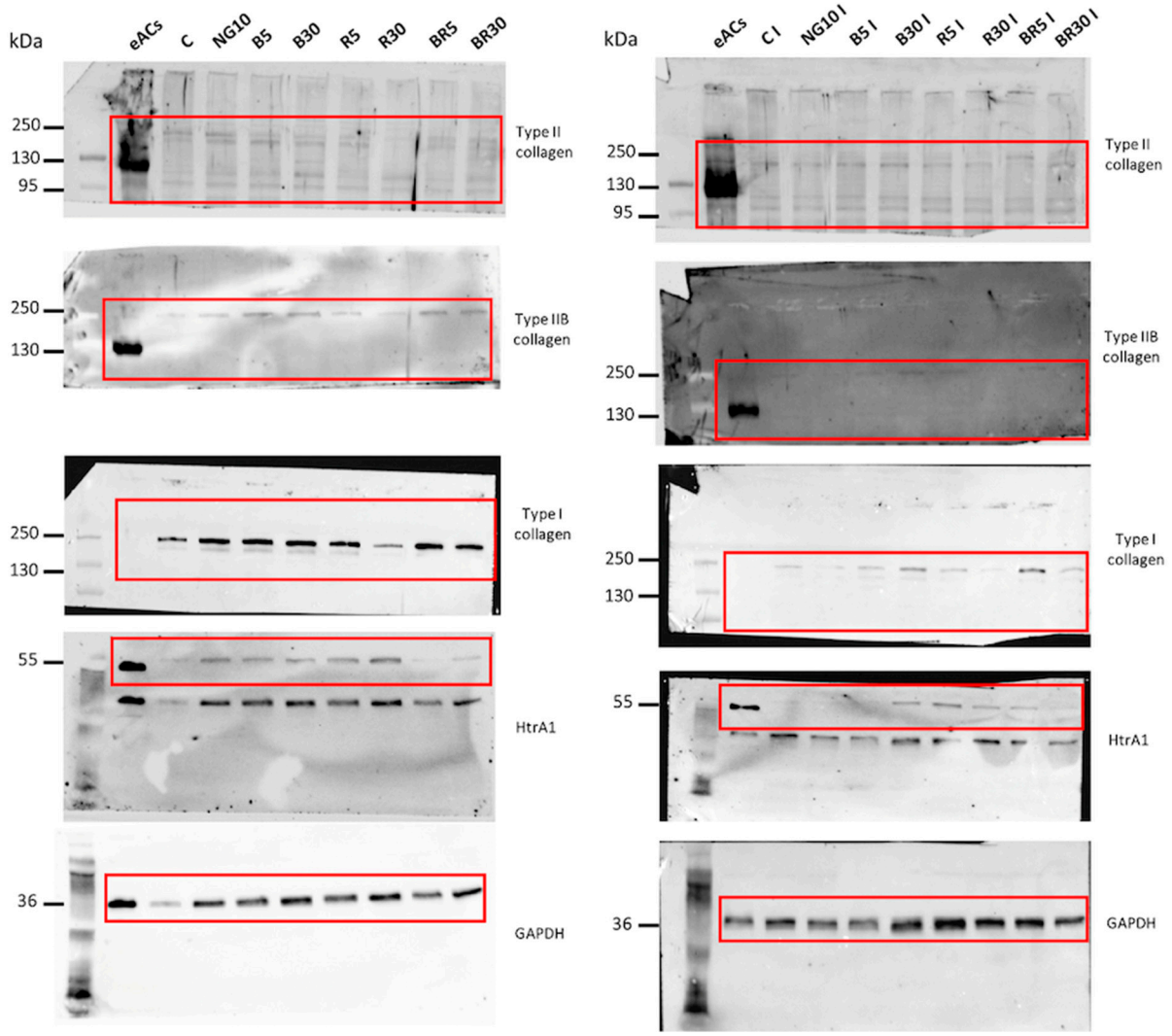


**Figure S5.** Complete gel and PVDF membranes analyzed in the western blots. For the western blots presented in Figure 9B and C, the entire images of the membranes captured with Chemidoc MP Imaging System (Bio-Rad) are shown. The molecular weight marker (MW, kDa) is indicated on the left side of the images shown. Red boxes indicate the cropped images shown in Figure 9B and C.

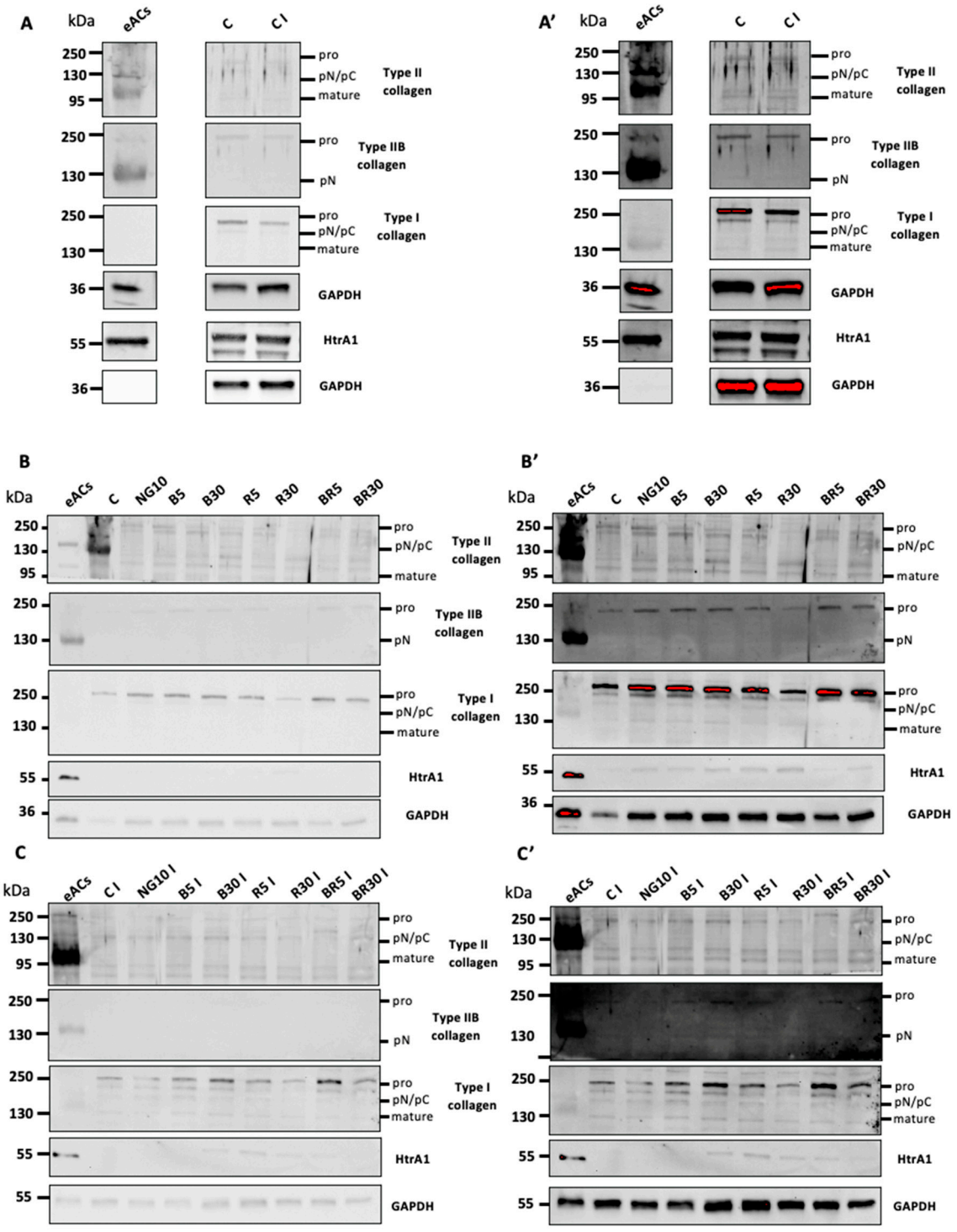


**Figure S6.** Under- and overexposure of the blots shown in Figure 9. Under-exposure (A, B and C) and over-exposure (A', B' and C') of blots shown in Figure 9A, B and C. The molecular weight marker (kDa) is indicated on the left side of the images.

**Supplementary Table S1.** Sequences of the primers used in RT-qPCR

| Gene | Forward sequence | Reverse sequence |
|---|---|---|
| ***ACAN*** | TGT CAA CAA CAA TGC CCA AGA C | CTT CTT CCG CCC AAA GGT CC |
| ***ACTB*** | GAT GAT GAT ATC GCC GCG CTC | TGC CCC ACG TAT GAG TCC TT |
| ***ADAMTS5*** | AAG GGA CAC CAT GTG GCA AA | CCC ACA TGA GCG AGA ACA CT |
| ***ALPL*** | GAC ATG ACC TCC CAG GAA GA | GCA GTG AAG GGC TTC TTG TC |
| ***BDKRB1*** | GGA GAC CAA TGT TCC TGT CTG T | TGG CAT TTG GAG GCA AGA GC |
| ***BGLAP*** | TCC TTT GGG GTT TGG CCT AC | GCC TGT GAG ACA AAG GAG GG |
| ***COL1A1*** | TGC CGT GAC CTC AAG ATG TG | CGT CTC CAT GTT GCA GAA GA |
| ***COL1A2*** | CCA GAG TGG AGC AGC GGT TA | GGG ATG TTT TCA GGT TGA GCC |
| ***COL2A1*** | GGC AAT AGC AGG TTC ACG TAC A | CGA TAA CAG TCT TGC CCC ACT T |
| ***COL10A1*** | GCA CCC CAG TAA TGT ACA CCT ATG | GAG CCA CAC CTG GTC ATT TTC |
| ***COL11A1*** | TTG CTG ATG GGA AGT GGC AT | GCT GCT TTG GGG TCA CCT AT |
| ***EDNRA*** | ATT TAA GCT GCT AGC TGG GC | ATC CCG ATT CCC TGA ACA CG |
| ***PTGS2*** | CGA GGT CCA GCT TTC ACC A | GCG GAT ACA CCT CGC CAT T |
| ***PRG4*** | CTA CCA CCC AAC GCA ACA AA | ACT GTT GTC TCC TTA TTG GGT |
| ***HTRA1*** | GGA CTT CAT GTT TCC CTC AA | GTT CTG CTG AAC AAG CAA CA |
| ***IL1B*** | CCC ACA CCA GTG ACA TGA TGA | TCC TCC TCA AAG AAC AGG TCA TTC |
| ***IL6*** | GGA TGC TTC CAATCT GGG TTC AAT | TCC GAA AGA CCA GTG GTG ATT TT |
| ***IL8*** | GCC ACA CTG CGA AAA CTC A | GCA CAA TAA TCT GCA CCC ACT TT |
| ***IL18*** | GAA ATC AAC CTG TGT TTG AGG ATA TG | TCA CAG AGA TGG TTA CCG CTA GAC |
| ***INOS*** | TTT GGC TGG TCC CCC GAT TT | GCC AGC GTT TCC GAT TTT CC |
| ***KI67*** | AAG CTG CAC GTT CAT GGA GA | ACC CAC AGT TCT TCC TCC GA |
| ***MMP1*** | CGA AGG GAA CCC TCG GTG GGA | TGG CCT GGT CCA CAT CTG CTC |
| ***MMP3*** | GAG GAA ATG AGG AAC AAG CGG | GAG GGA AAC CCA GAG TGT GGA |
| ***MMP13*** | TGA AGA CCC GAA CCC TAA ACA T | GAA GAC TGG TGA TGG CAT CAA G |
| ***P53*** | CAC CTG AGG TTG GCT CTG AC | GCA CAA ACA CGC ACC TCA AA |
| ***P65*** | CAC GGA TAC CAC CAA GAC CC | GTC TGG ATG CGC TGA CTG AT |
| ***SOX9*** | CAA GAA GGA CCA CCC GGA CTA | GGA GAT GTG TGT CTG CTC CGT |
| ***SPP1*** | GAG ACA CGT ATG ATG GCC GA | GCT GTC CCA ATC AGA AGC CA |
| ***TUBA*** | GAG TTT TCT GAG GCC CGT GA | GTC TCC CTG TAA AAG CAG CAC |

**Supplementary Table S2.** Antibodies used in the western blots

| Antibody | Supplier | Dilution |
|---|---|---|
| **Type IIB collagen** | Covalab, Villeurbanne, France | 1:750 |
| **Type II collagen** | Novotec, Bron, France | 1:750 |
| **Type I collagen** | Novotec, Bron, France | 1:3000 |
| **HtrA1** | ABGENT, San Diego CA, USA | 1:3000 |
| **GAPDH** | Santa Cruz Biotechnology, Dallas TX, USA | 1:3000 |