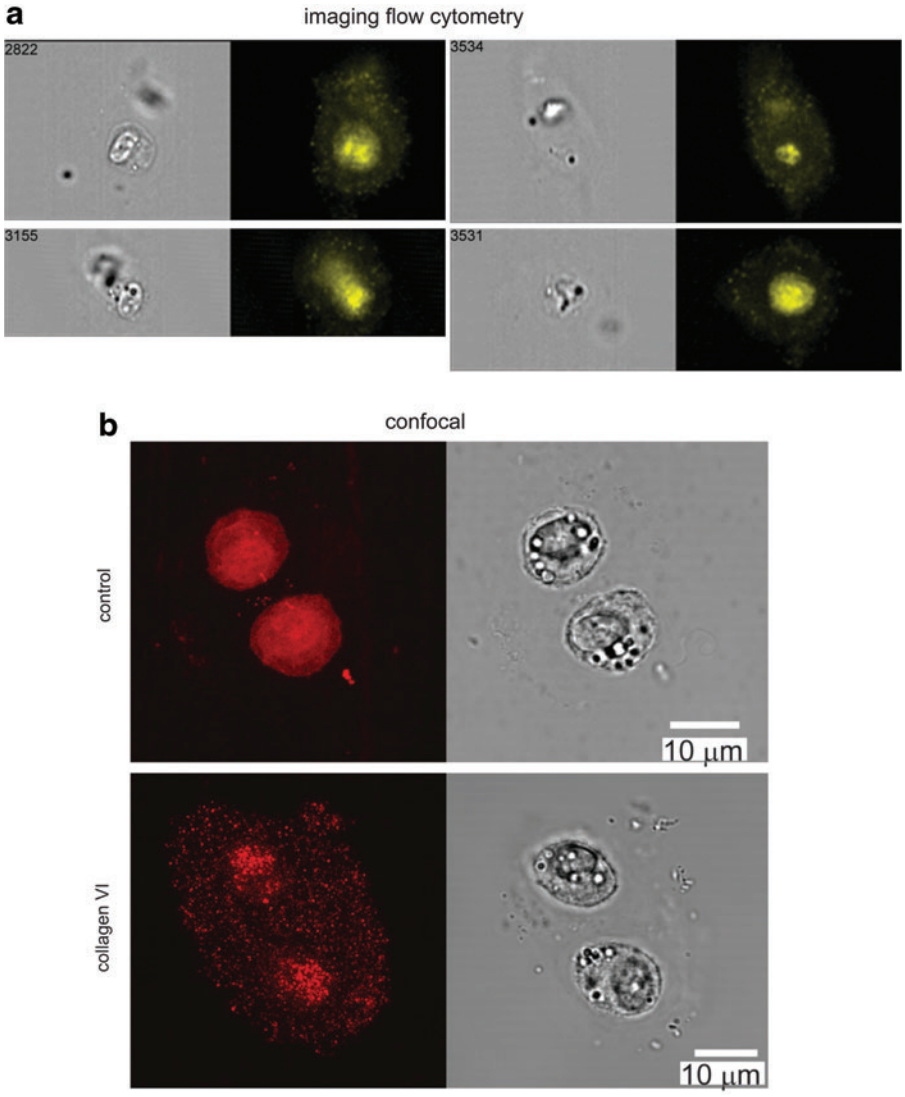
Supplementary Data

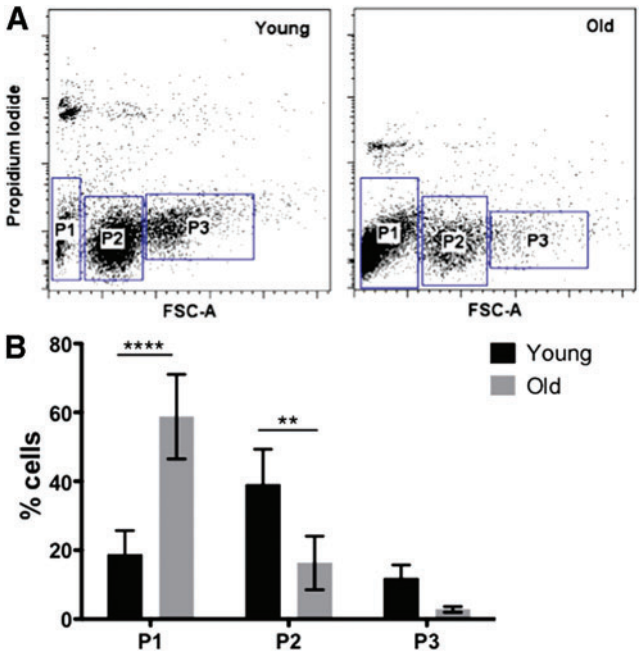
Supplementary Materials and Methods

Collagen type VI staining

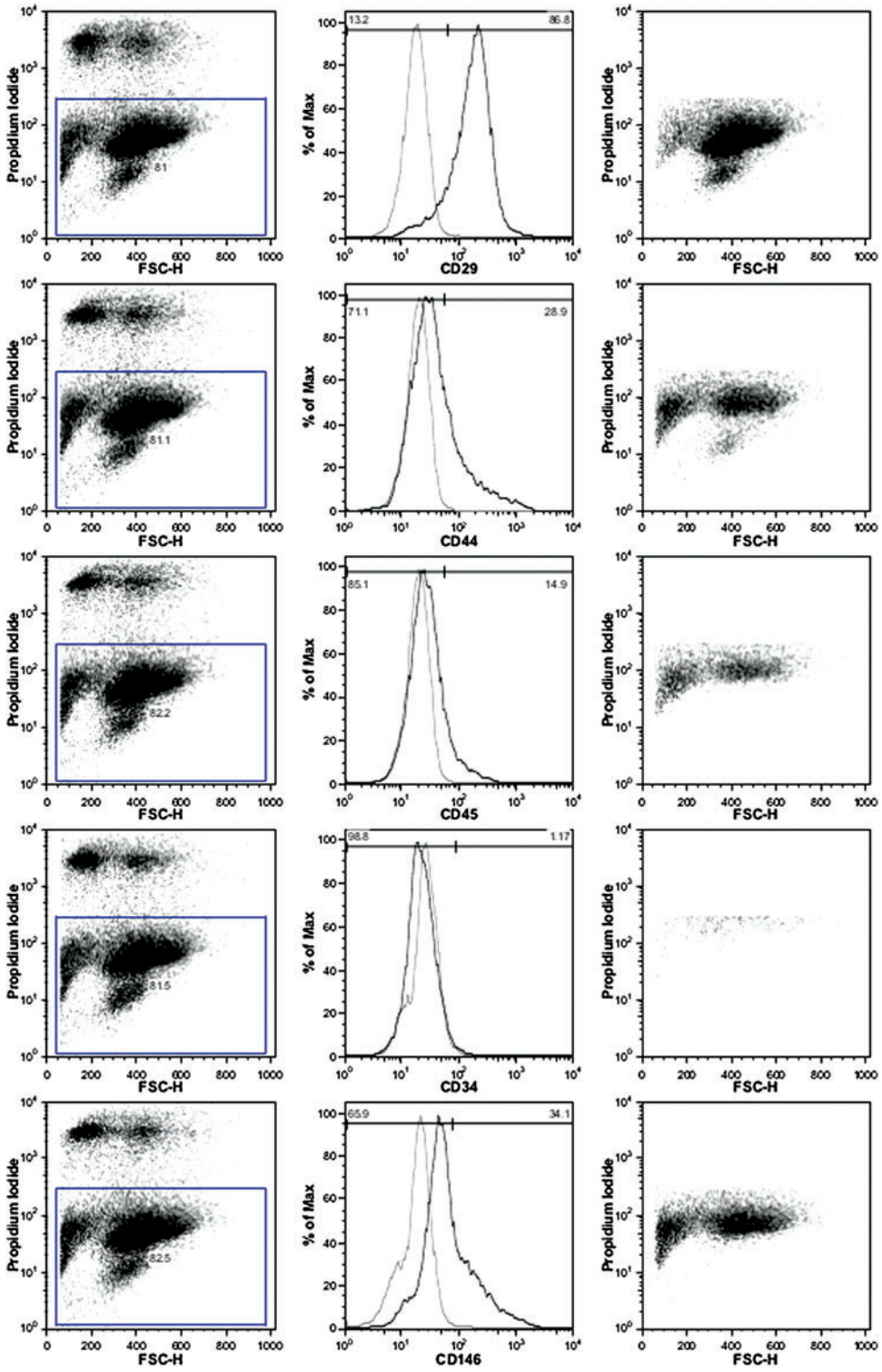
Freshly isolated nucleus pulposus (NP) cells were permeabilized by incubating them for 5 min with phosphate-buffered saline (PBS)/0.2% v/v Triton and blocked by incubating them with PBS/bovine serum albumin (BSA) 3% for another 5 min on ice. Cells were then stained by incubating them with an anti-collagen VI antibody (Santa Cruz; sc-20649) at 10 μg/mL in PBS/BSA 3% for 1.5 h on ice. Cells were then washed thrice with PBS and incubated with the secondary antibody Cy3 conjugated anti-rabbit IgG (Jackson Immunoresearch) at 1:500 in PBS/BSA 3% for 1 h on ice. Cells were again washed twice with PBS and fixed by incubating them for 15 min at room temperature with 4% paraformaldehyde. Finally, cells were washed twice with PBS before imaging. For imaging flow cytometry, cells were filtered through a 100 μm mesh before analysis on an ImageStreamx equipped with a 488 nm laser, as described in the main text. Cells were visualized in channels 1 (brightfield) and 3 (Fluorescence staining). For confocal imaging, 4 μL of cell suspension were placed between a slide and a coverslip. Cells were then imaged with a 40× oil objective with a Leica TCS SP5 II laser scanning confocal microscope (Leica Microsystems).



SUPPLEMENTARY FIG. S1. Expression of type VI collagen on the pericellular matrix of bovine nucleus pulposus (bNP) cells. Micrographs show brightfield (*left*) and fluorescence images (*right*) of freshly isolated bNP cells stained against collagen type VI and imaged by imaging flow cytometry **(a)** or confocal microscopy **(b)**. A punctuated staining can be observed located in and outside the cytoplasm of the cells. Fluorescence and brightfield in **(b)** show a maximum projection and single slice, respectively. Control refers to cells stained only with the secondary antibody.



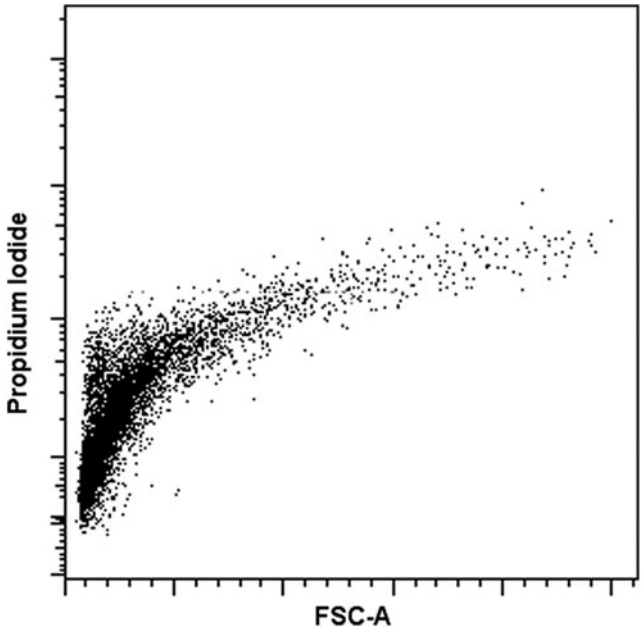
SUPPLEMENTARY FIG. S2. Comparison of size of bNP cells and auto-fluorescence analyzed by flow cytometry of young and aged animals. **(A)** Representative dot plots of propidium iodide expression versus size (FSC) of bNP cells from young and old animals. **(B)** Distribution of P1, P2, and P3 subpopulations in young and old bovine animals. Results are presented as mean ± SD ($n = 5$ for old animals, $n = 4$–7 for young animals) and were analyzed for statistical differences using non-parametric Kruskal–Wallis and Dunn's multiple-comparison tests (**$0.001 < p < 0.01$ and ****$p < 0.0001$).



SUPPLEMENTARY FIG. S3. Representative analysis of the distribution of each analyzed marker (CD29, CD44, CD45, CD34, and CD146) within the three populations (P1, P2, and P3) of bNP cells.

Isolation of human NP cells

Human NP biopsies were isolated from lumbar intervertebral discs (IVDs) in microdiscectomy surgeries of patients with IVD pathology (that may include disc herniation, degenerative disc disease, spinal stenosis, or spondylolisthesis) at Centro Hospitalar S. João under approval of the Hospital Ethics Committee and after patients' informed consent. Lumbar IVDs with grade III or IV according to the Pfirrmann scale were isolated, but only contained fragments of NP were used in this study. Human NP fragments were digested and processed as described for bovine NP.



SUPPLEMENTARY FIG. S4. Representative dot plot of propidium iodide expression versus size (FSC) of human NP cells obtained from biopsies of degenerated intervertebral disc with contained NP.