



## Supplementary Material

10.1302/2046-3758.123.BJR-2022-0199.R1

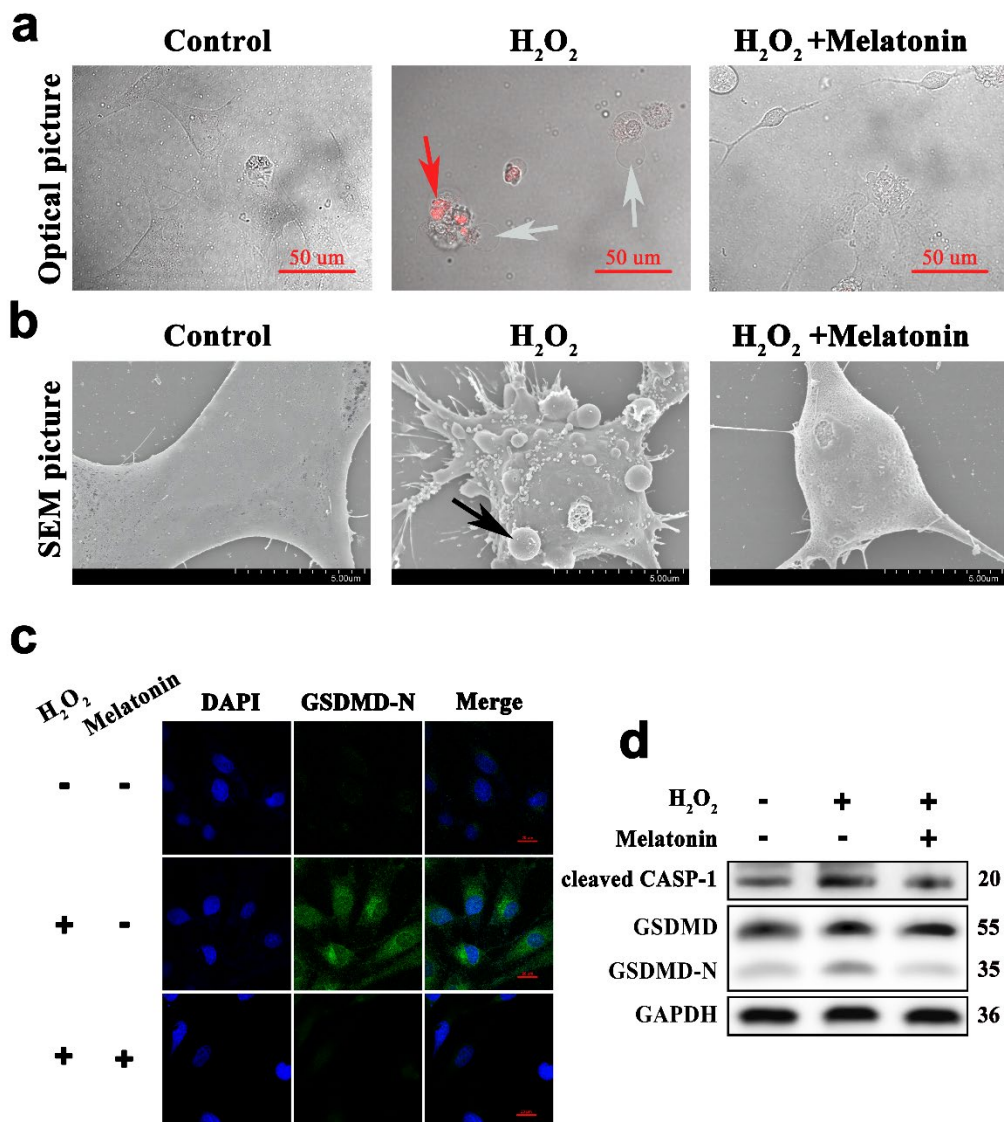
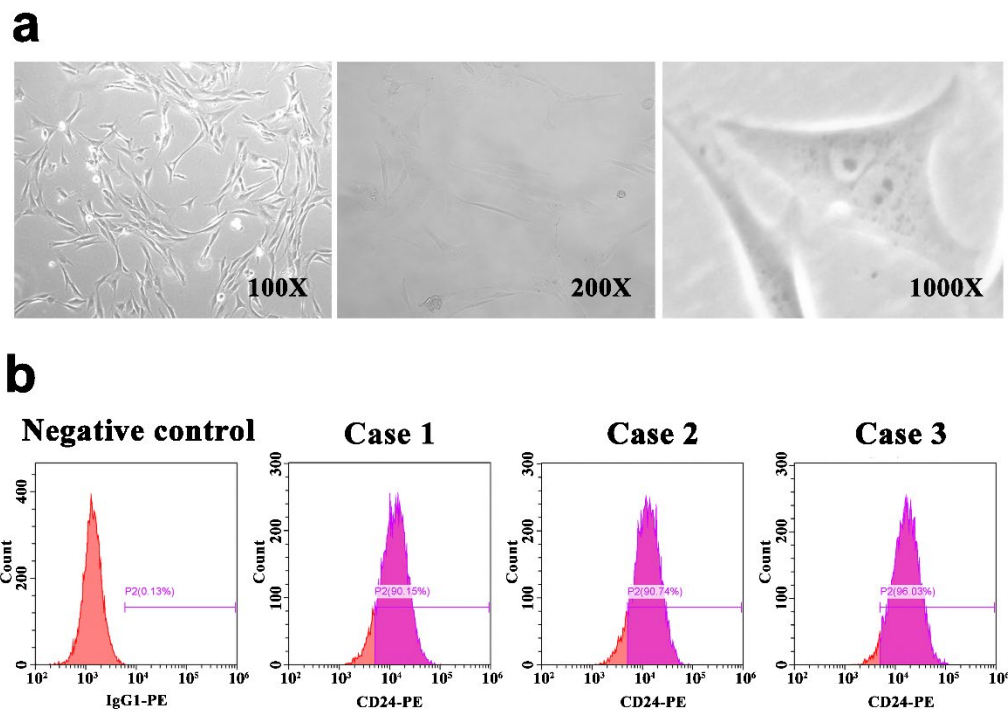
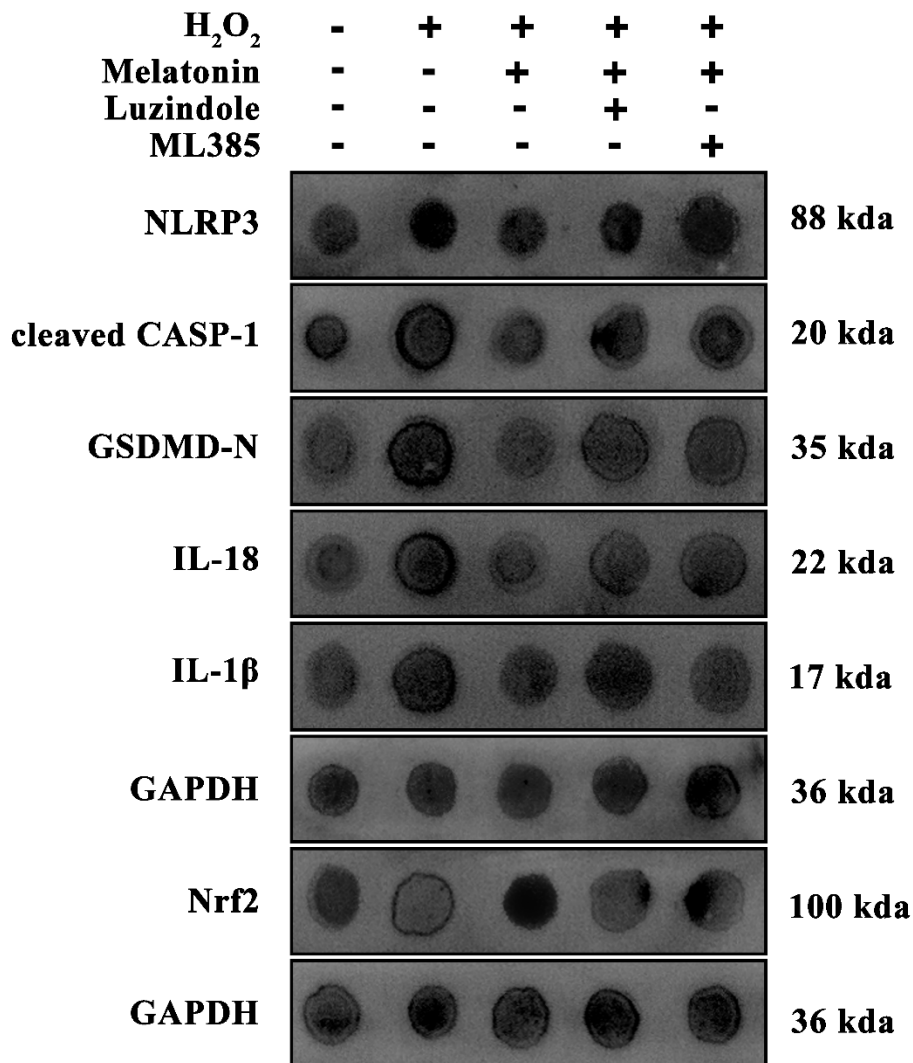


Fig a. The pyroptosis process of nucleus pulposus cells (NPCs) was observed by a) optical microscopy and b) scanning electron microscopy (SEM), respectively.

The expression of gasdermin N-terminal (GSDMD-N), the executive protein of pyroptosis, was detected by c) immunofluorescence and d) Western blot. The grey arrows indicate the vesicular projection (a characteristic feature of pyroptosis); the red arrow indicates the NPCs positive with propidium iodide staining; the black arrows indicate the pyroptosis body. CASP-1, caspase-1; DAPI; 4',6-diamidino-2-phenylindole; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.



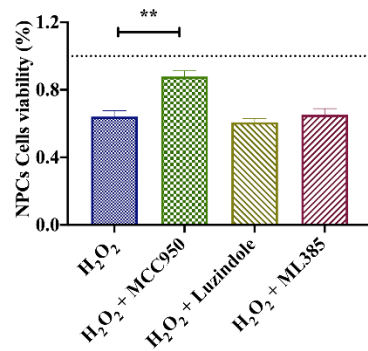
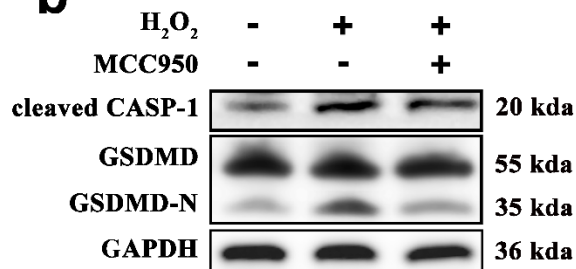
**Fig b.** a) Observation of nucleus pulposus cell (NPC) morphology by optical microscopy. NPCs were identified with the fluorescently labelled monoclonal antibody CD24. b) The proportion of fluorescent cells was measured through flow cytometry.



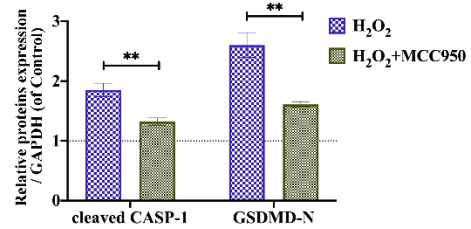
**Fig c.** Dot blot results of pyroptosis-related proteins in nucleus pulposus cells treated with different drugs. CASP-1, caspase-1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GSDMD-N, gasdermin N-terminal; IL, interleukin; NLRP3, pyroptosis-related proteins NLR family pyrin domain containing 3; Nrf2, nuclear factor erythroid 2-related factor 2.

**a**

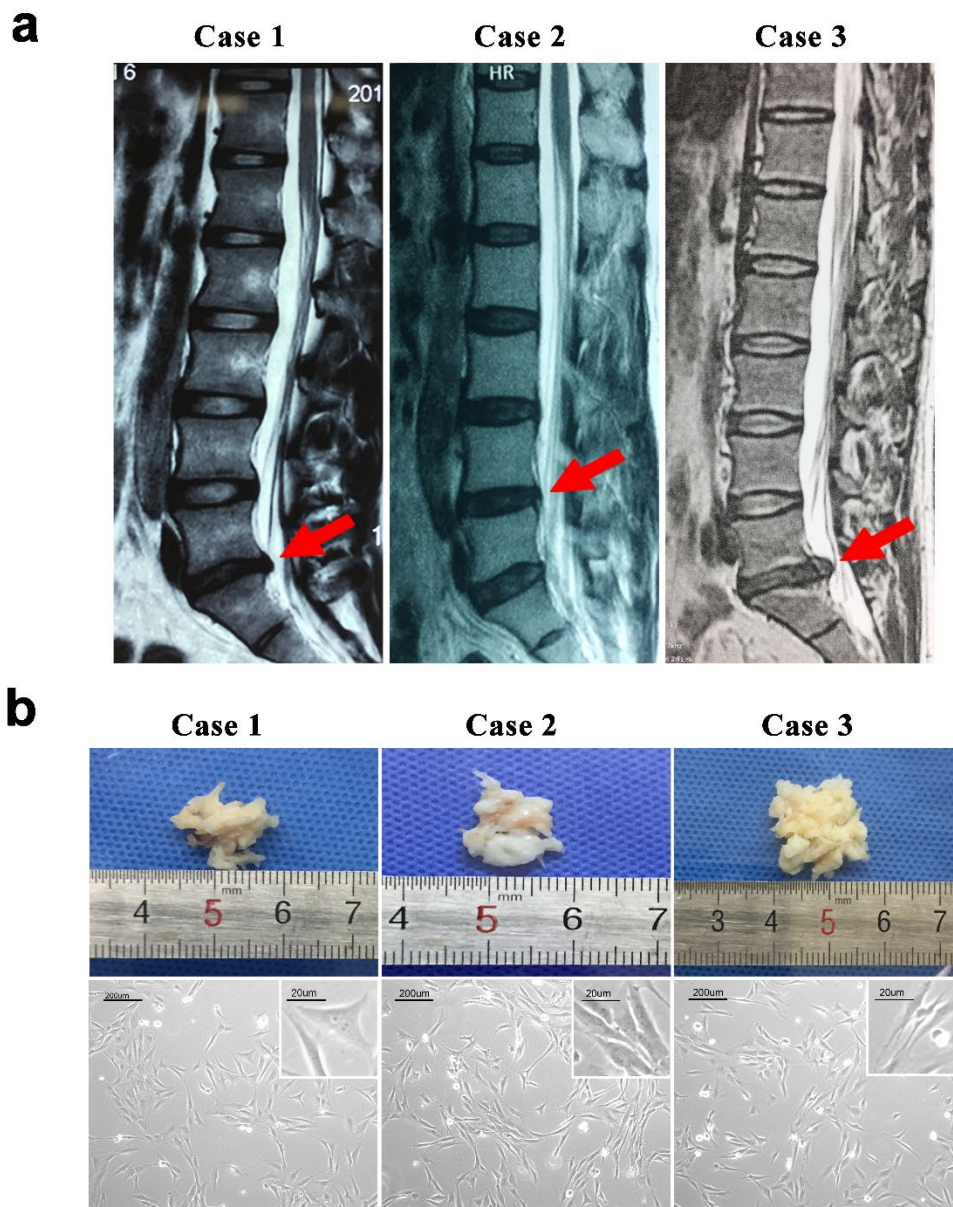
The cell viability of NPCs treated with different drugs

**b****c**

The expression of pyroptosis related proteins of NPCs treated with MCC950



**Fig d.** Pyroptosis-related indexes of nucleus pulposus cells (NPCs) treated with different drugs. a) Cell counting kit-8 results of NPCs treated with different drugs. b) Western blot was used to detect the expression of pyroptosis-related proteins in NPCs treated with different drugs. c) The panel shows greyscale histograms of Western blot analysis. \* $p < 0.05$ , \*\* $p < 0.01$ . CASP-1, caspase-1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GSDMD-N, N-terminal fragment of gasdermin D.



**Fig e.** Identification of nucleus pulposus cells (NPCs) isolated from patients with lumbar disc herniation (LDH). a) The MRIT2 weighted images show the intervertebral disc of the patients with LDH. Black arrows indicate the herniated disc. b) Macroscopic observation of nucleus pulposus tissues and microscopic observation of the cultured NPCs (magnification:  $\times 100$ , scale bars = 200  $\mu\text{m}$ ).