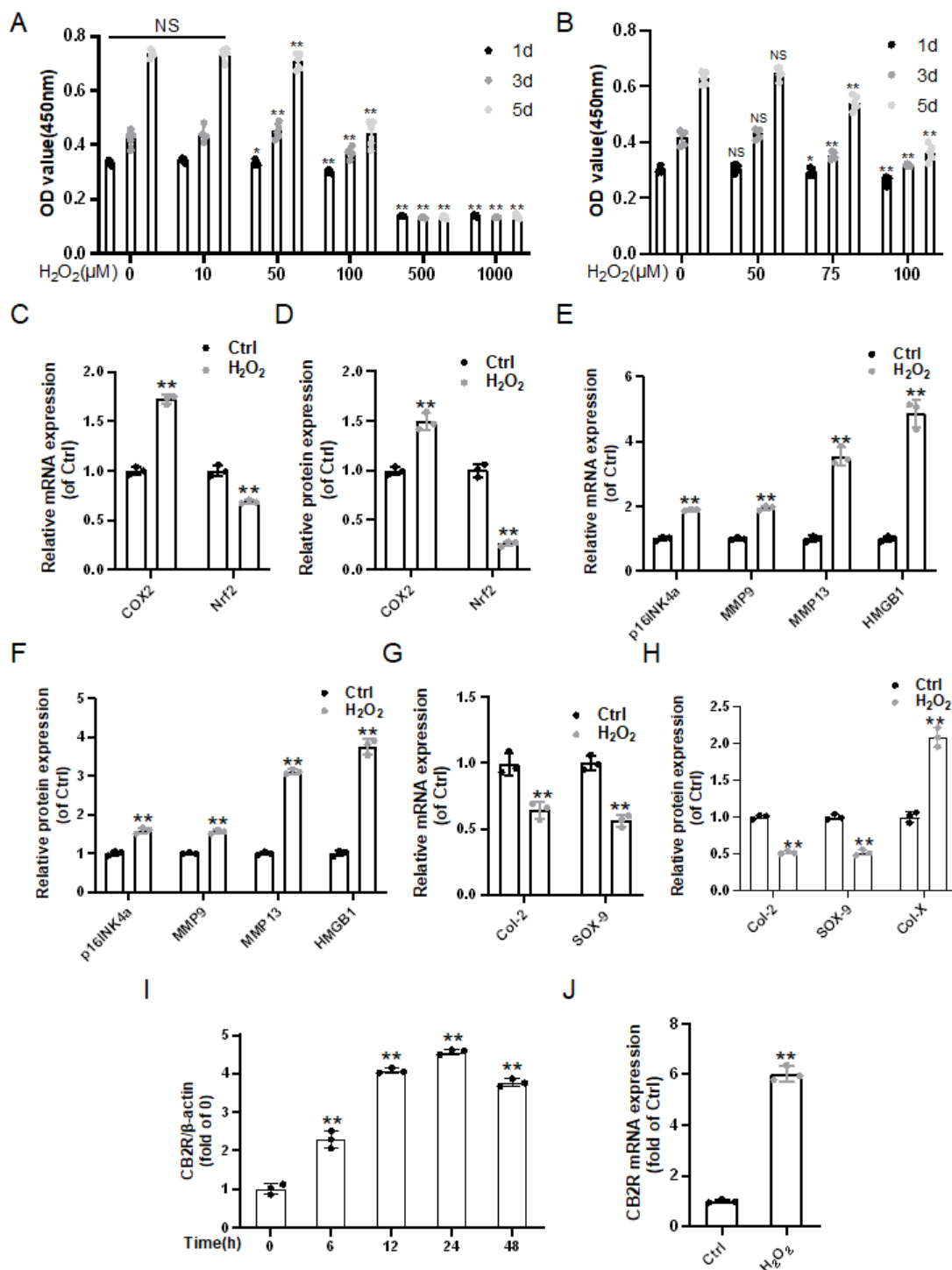


CB2R Attenuates Intervertebral Disc Degeneration by Delaying Nucleus Pulposus Cell Senescence through AMPK/GSK3 β Pathway

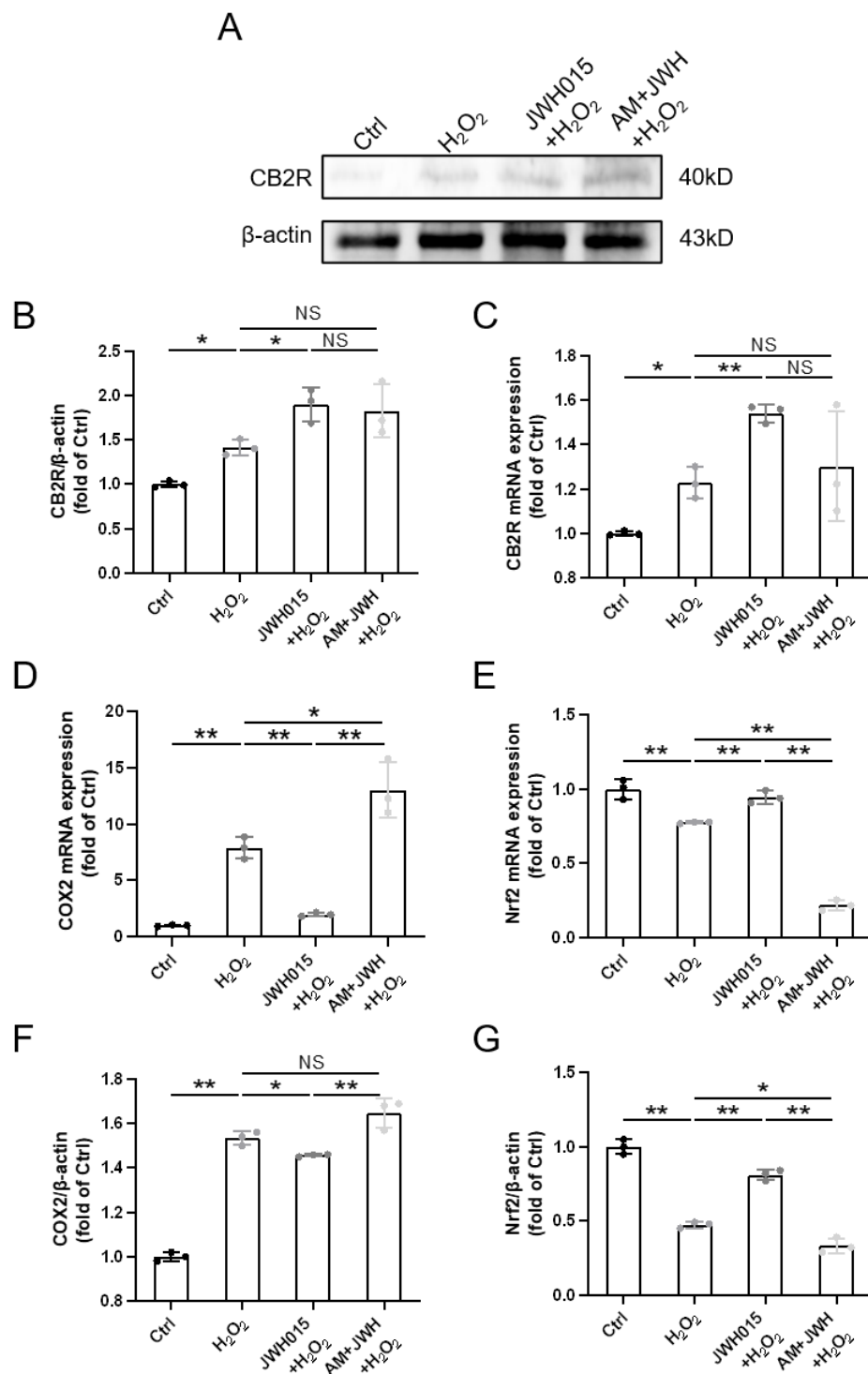
Jiacheng Du^{1,*}, Menglei Xu^{2,*}, Fanchen Kong^{1,*}, Pengfei Zhu^{1,*}, Yubo Mao¹, Yijie Liu¹, Hong Zhou¹, Zhongchen Dong¹, Zilin Yu¹, Tong Du³, Ye Gu⁴, Xiexing Wu¹, Dechun Geng¹, Haiqing Mao¹

SUPPLEMENTARY DATA



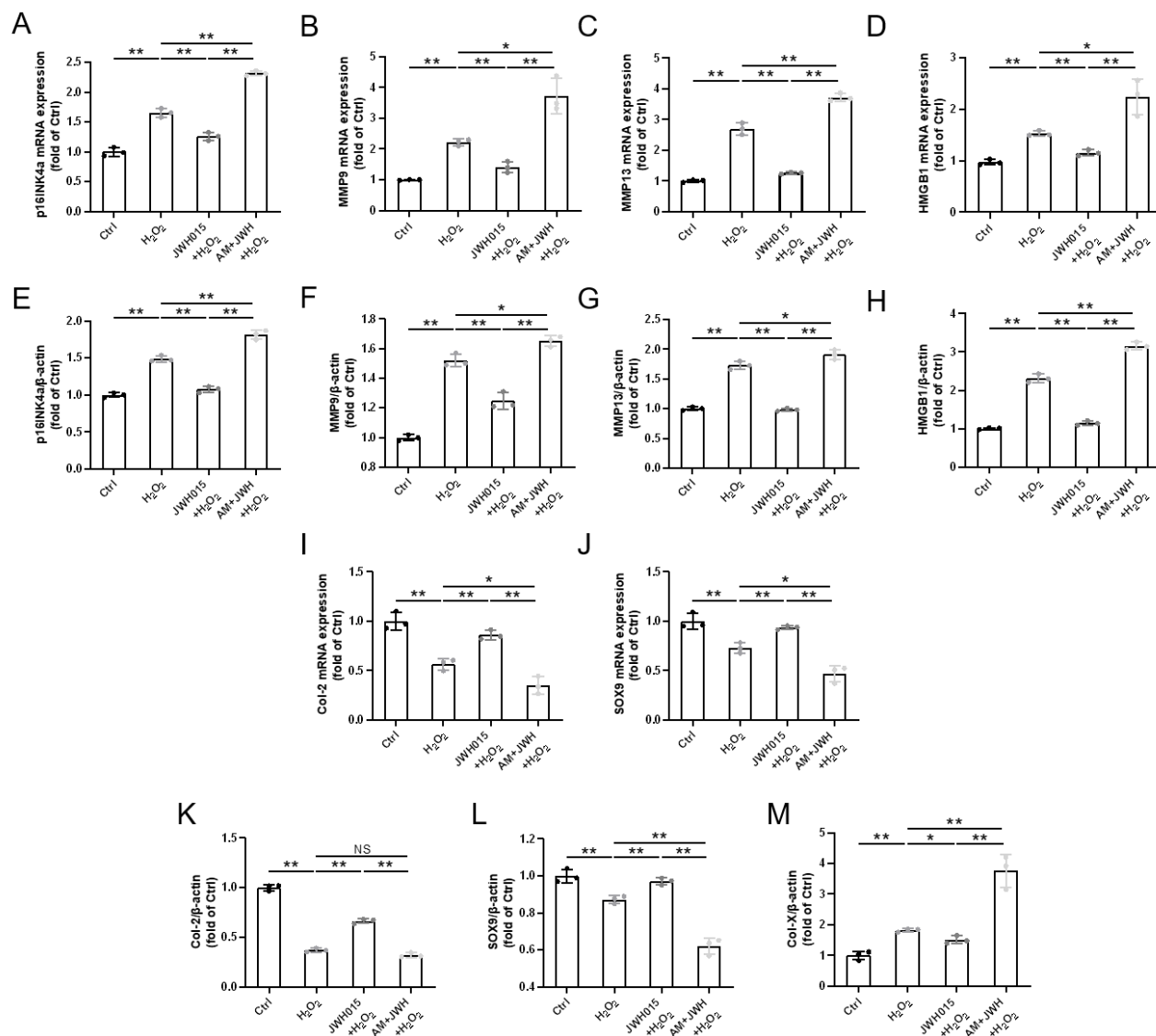
Supplementary Figure 1. (A) Effects of different concentrations (0, 10, 50, 100, 500 and 1000 μM) of H_2O_2 on the proliferation of NPC; (B) Effects of different concentrations (0, 50, 75 and 100 μM) of H_2O_2 on the proliferation of NPC; (C) RT-PCR results of COX2 and Nrf2; (D) WB results of COX2 and Nrf2; (E) The mRNA level of p16INK4a, MMP9, MMP13 and HMGB1; (F) The protein level of p16INK4a, MMP9, MMP13 and HMGB1; (G) RT-PCR results of Col-2 and SOX9; (H) Western blot quantitative results of Col-2, SOX9 and Col-X; (I) Western blot quantitative results of time-dependent of CB2R expression under oxidative stress; (J) RT-PCR results of CB2R. All experiments were performed thrice, and the data are shown as the mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$.

SUPPLEMENTARY DATA



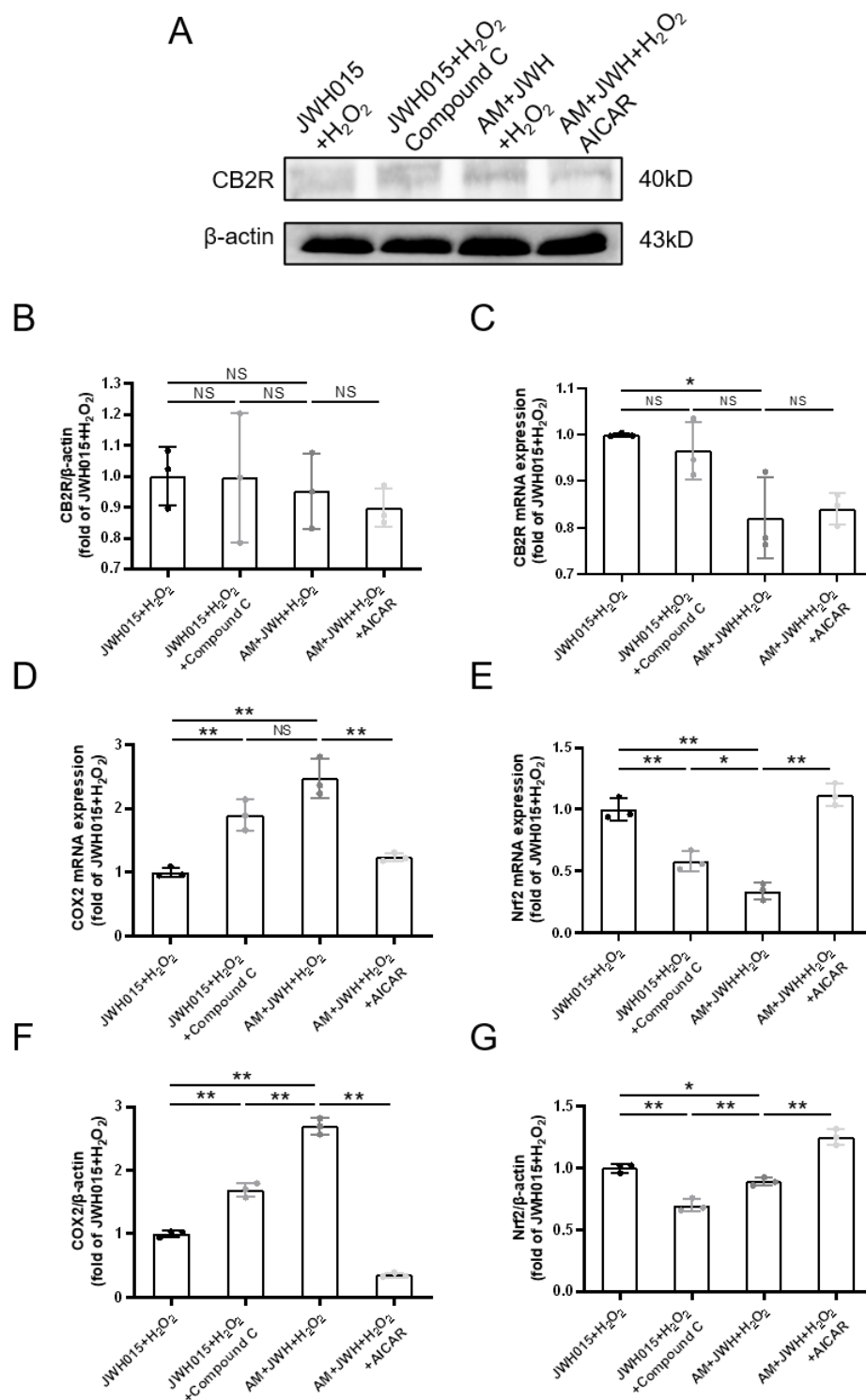
Supplementary Figure 2. (A-B) Western blot results of CB2R; **(C)** RT-PCR results of CB2R; **(D-E)** Western blot quantitative results of COX2 and Nrf2; **(F-G)** The mRNA level of COX2 and Nrf2. All experiments were performed thrice, and the data are shown as the mean $\pm$ SD. * p < 0.05, ** p < 0.01.

SUPPLEMENTARY DATA



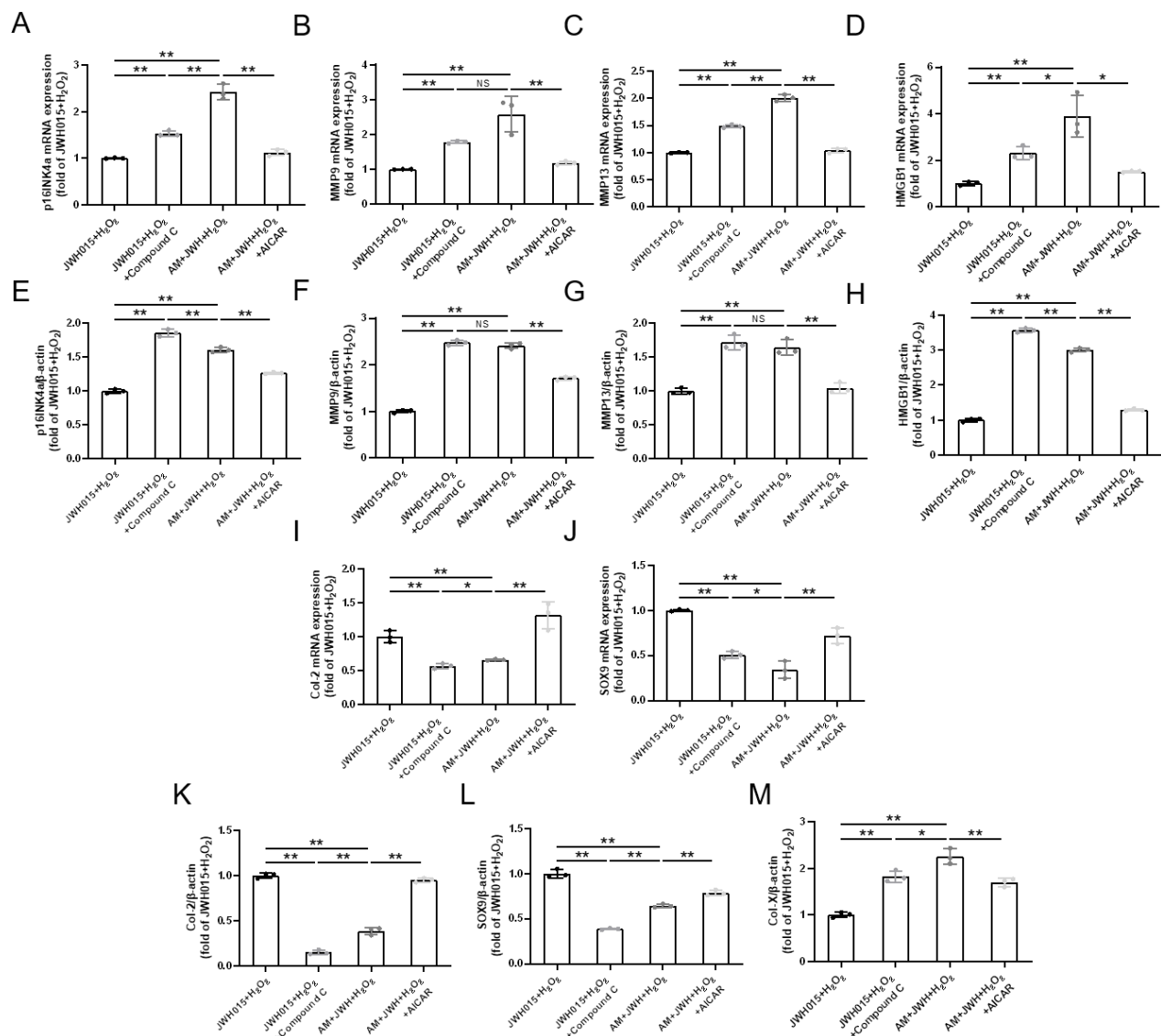
Supplementary Figure 3. (A-H) The mRNA and protein expression levels of p16INK4 α , MMP9, MMP13 and HMGB1; (I-M) The RT-PCR and western blot results of Col-2, SOX9 and Col-X. All experiments were performed thrice, and the data are shown as the mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$.

SUPPLEMENTARY DATA



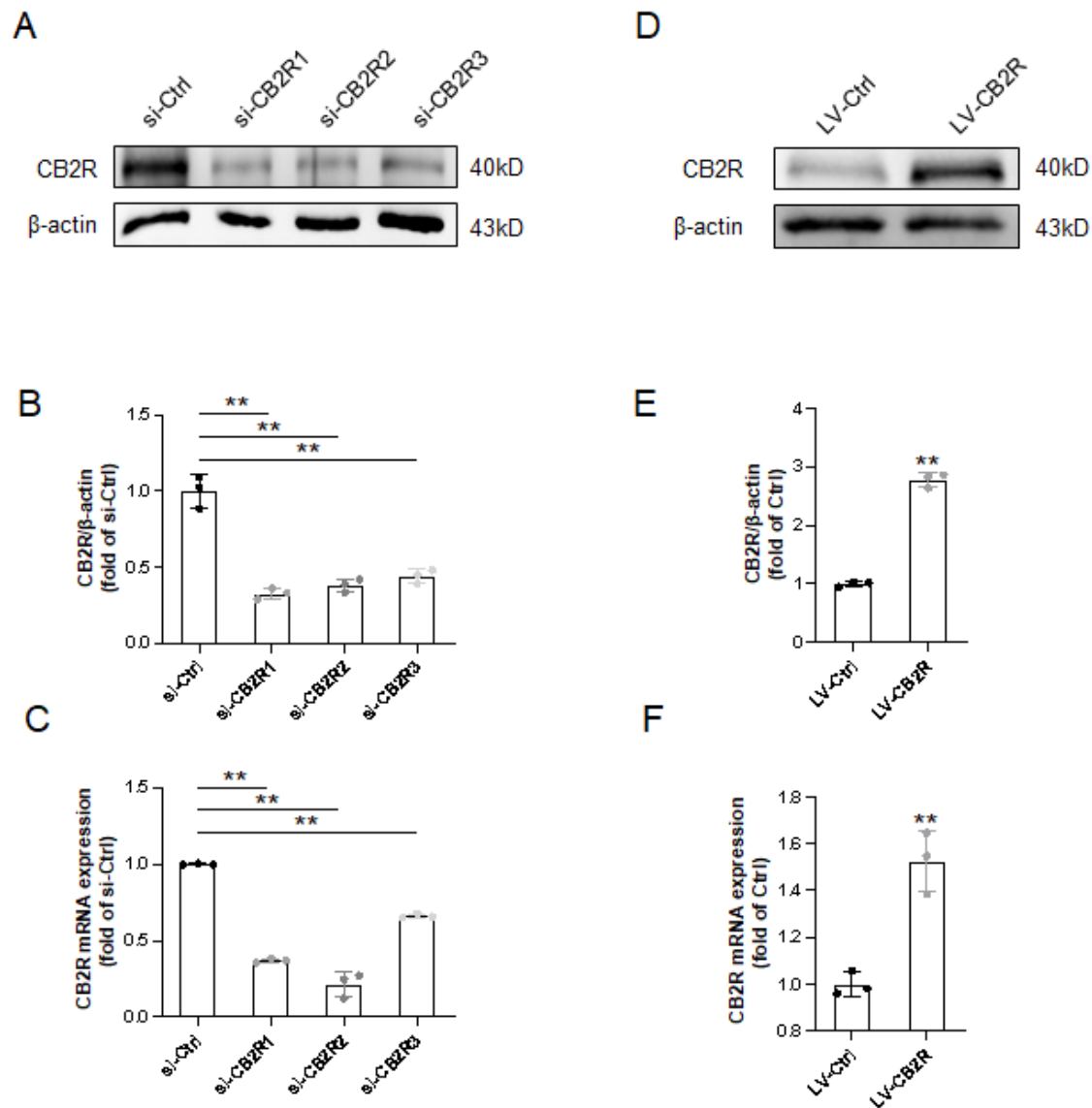
Supplementary Figure 4. (A-B) Western blot results of CB2R; (C) RT-PCR results of CB2R; (D-G) The mRNA and protein expression levels of COX2 and Nrf2. All experiments were performed thrice, and the data are shown as the mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$.

SUPPLEMENTARY DATA



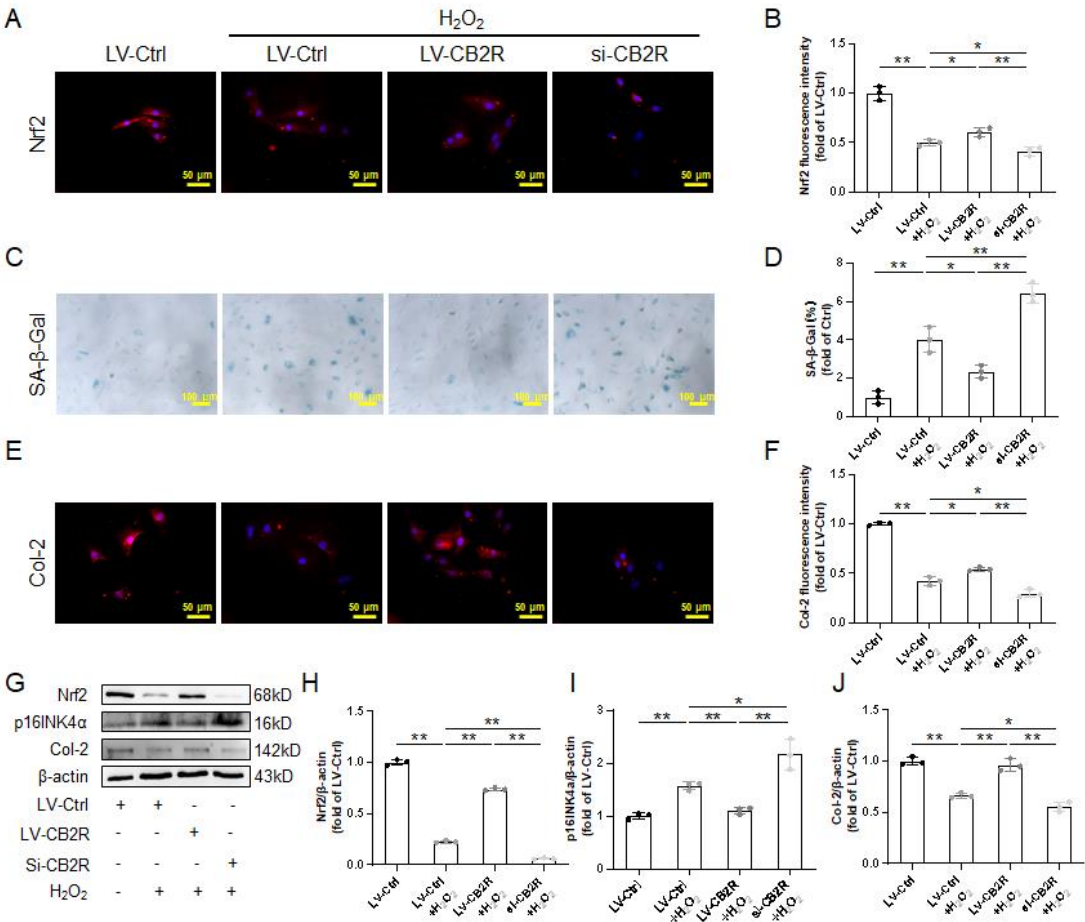
Supplementary Figure 5. (A-H) The mRNA and protein expression levels of p16INK4α, MMP9, MMP13 and HMGB1; **(I-M)** The RT-PCR and western blot results of Col-2, SOX9 and Col-X. All experiments were performed thrice, and the data are shown as the mean ± SD. **p* < 0.05, ***p* < 0.01.

SUPPLEMENTARY DATA



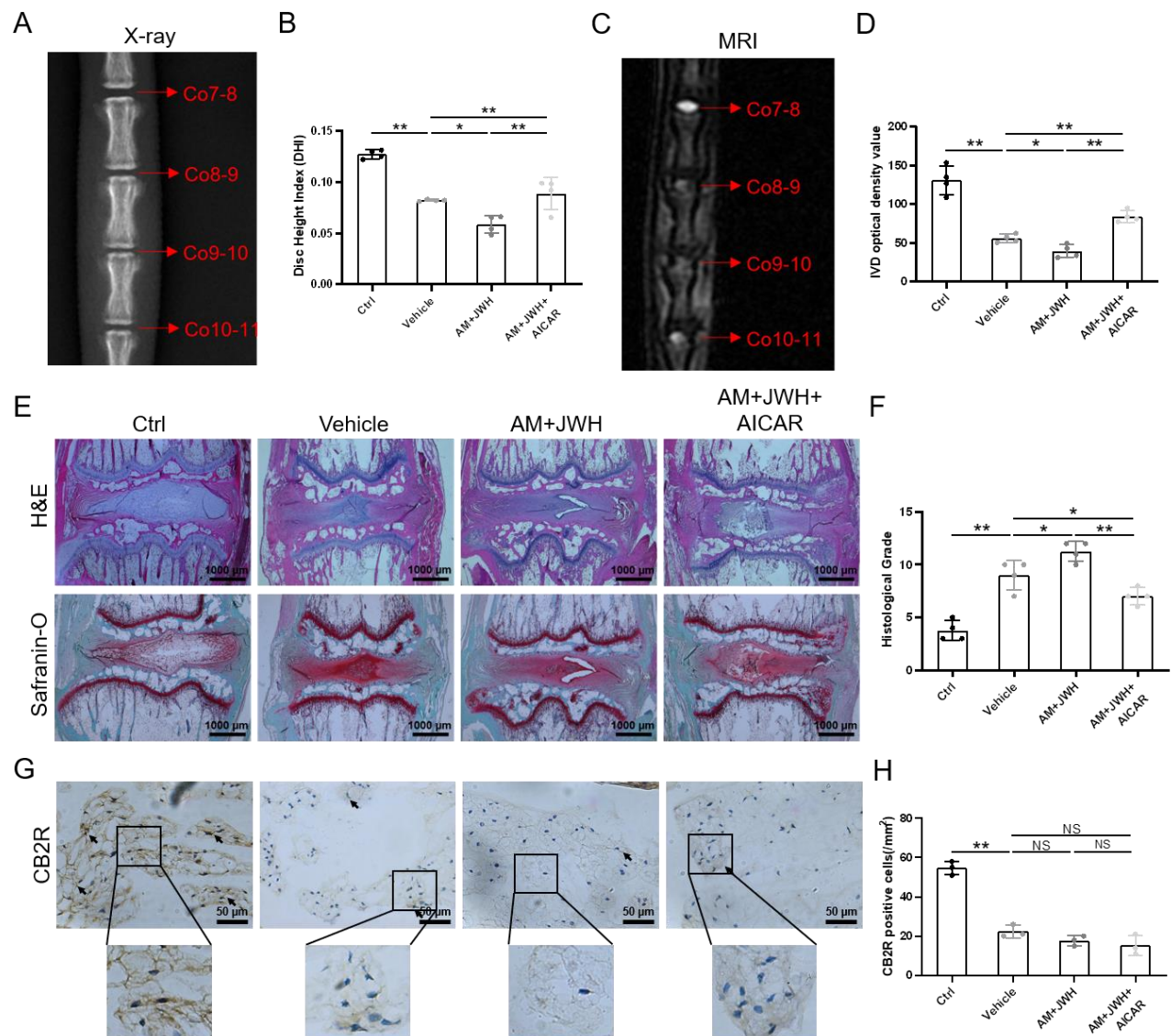
Supplementary Figure 6. (A-C) The western blot and RT-PCR results of CB2R siRNA transfection; (D-F) The western blot and RT-PCR results of CB2R lentivirus transfection. All experiments were performed thrice, and the data are shown as the mean $\pm$ SD. ** $p < 0.01$.

SUPPLEMENTARY DATA



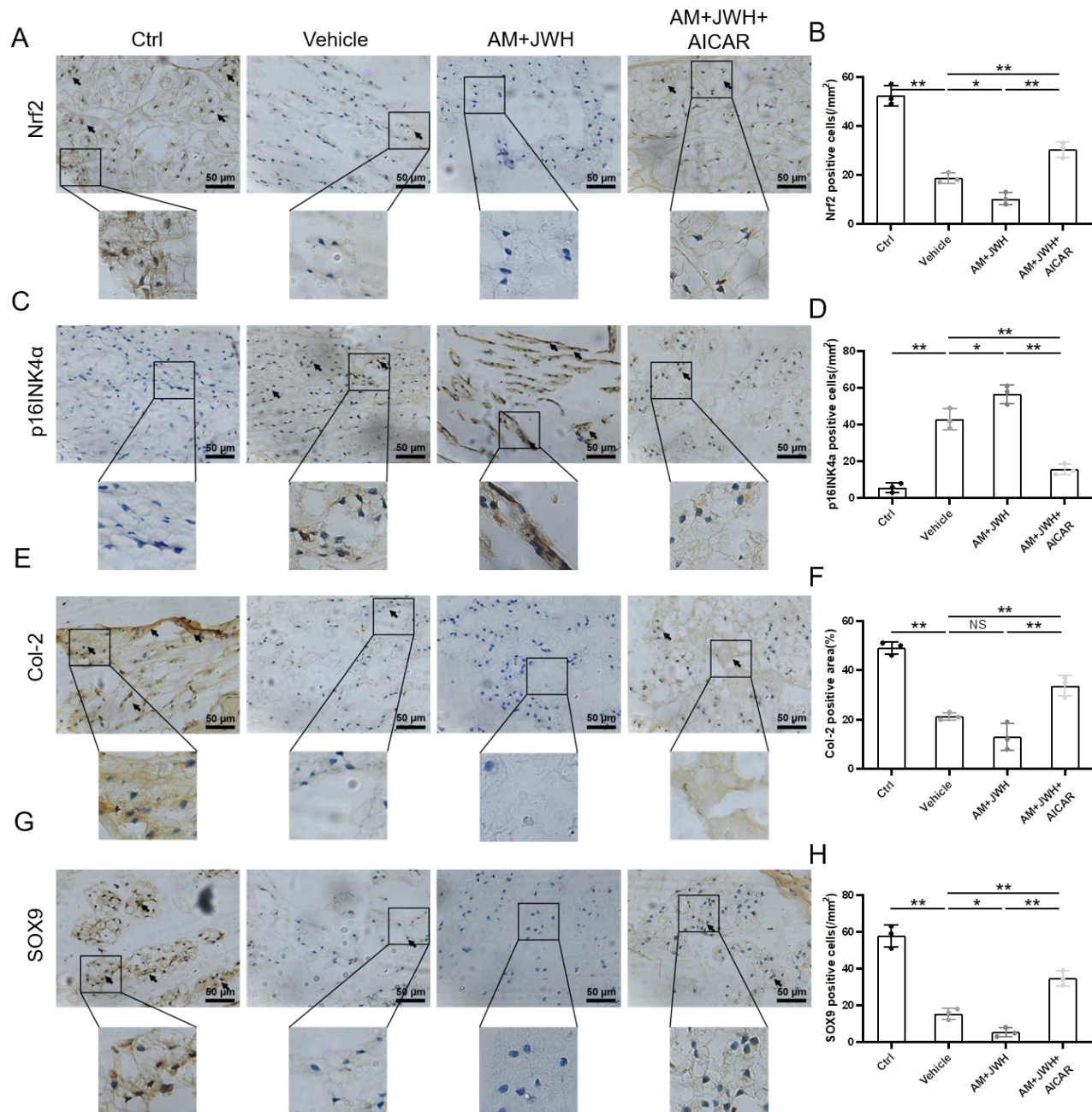
Supplementary Figure 7. (A-B) Immunofluorescence staining results of Nrf2 (scale bar = 50 μ m); **(C-D)** SA- β -Gal staining and quantitative analysis results (scale bar = 100 μ m); **(E-F)** Immunofluorescence staining results of Col-2 (scale bar = 50 μ m); **(G-J)** Western blot results of Nrf2, p16INK4 α , Col-2. Each experiment was conducted thrice, and the data are shown as the mean $\pm$ SD. * p < 0.05, ** p < 0.01.

SUPPLEMENTARY DATA



Supplementary Figure 8. (A) X-ray examination of rat caudal at 1 week after surgery. (red arrows indicate different IVDs); (B) Disc height index (DHI) of rat caudal; (C) T2-weighted MRI scans of rat caudal at 1 week after surgery. (red arrows indicate different IVDs); (D) The optical density value of IVD; (E) H&E and safranin-O staining of rat caudal IVD (scale bar = 1000 μ m); (F) Histological grade from the H&E and safranin-O staining; (G) Immunohistochemical staining results of CB2R (scale bar = 50 μ m, black arrows indicate positive regions or cells); (H) Quantitation of CB2R expression in rat caudal IVD. Each experiment was conducted at least thrice, * p < 0.05, ** p < 0.01.

SUPPLEMENTARY DATA



Supplementary Figure 9. (A) Immunohistochemical staining results of Nrf2 (scale bar = 50 μ m, black arrows indicate positive cells); (B) Quantification of Nrf2 expression in rat caudal IVD; (C) Immunohistochemical staining results of p16INK4a (scale bar = 50 μ m, black arrows indicate positive cells); (D) Quantification of p16INK4a expression in rat caudal IVD; (E) Immunohistochemical staining results of Col-2 (scale bar = 50 μ m, black arrows indicate positive cells); (F) Quantification of Col-2 expression in rat caudal IVD; (G) Immunohistochemical staining results of SOX9 (scale bar = 50 μ m, black arrows indicate positive cells); (H) Quantification of SOX9 expression in rat caudal IVD. * p < 0.05, ** p < 0.01.