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RESEARCH ARTICLE

3D-bioprinted kartogenin-laden hydrogel promotes cartilage regeneration via Smad1/5/9-mediated chondrogenesis of bone marrow stromal cells

Supplementary file

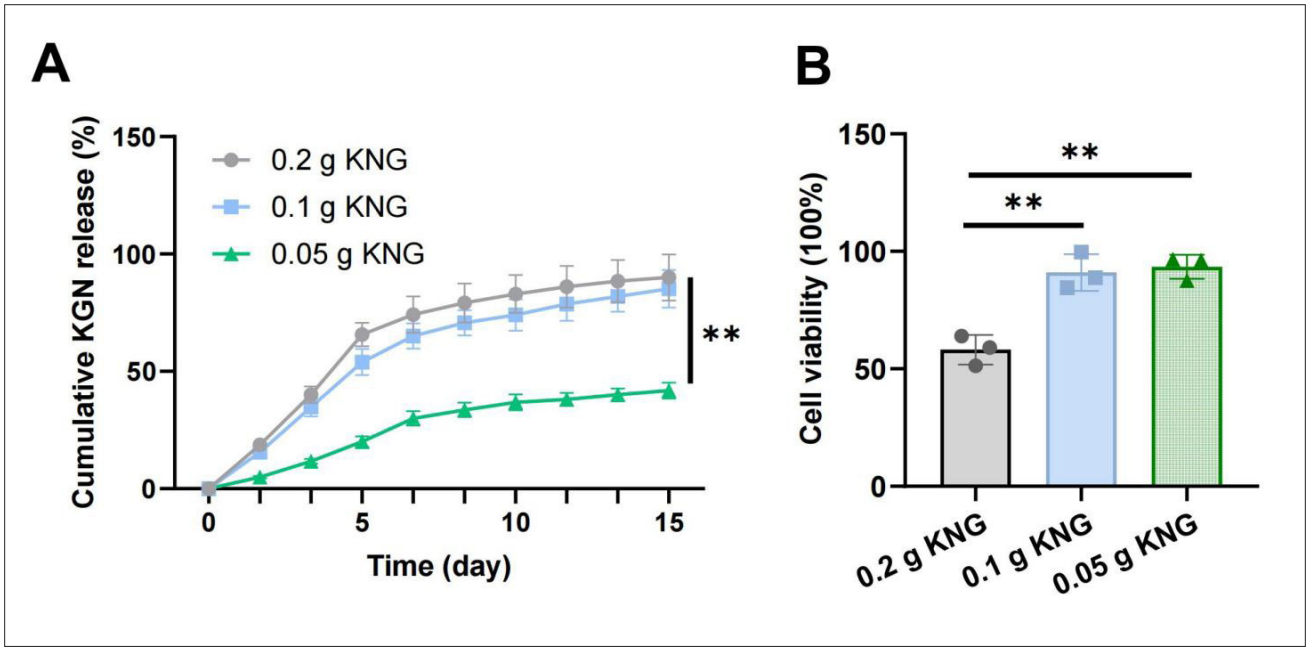

Figure S1. Analysis of KGN release gradient concentrations. (A) KGN release profile of Hy@KGN hydrogel in a simulated body fluid environment. (B) CCK-8 assay evaluating the cytotoxicity of each treatment on BMSCs. $^{**}p < 0.01$; $n = 3$. Abbreviations: BMSCs, bone marrow-derived mesenchymal stem cells; KGN, Kartogenin.

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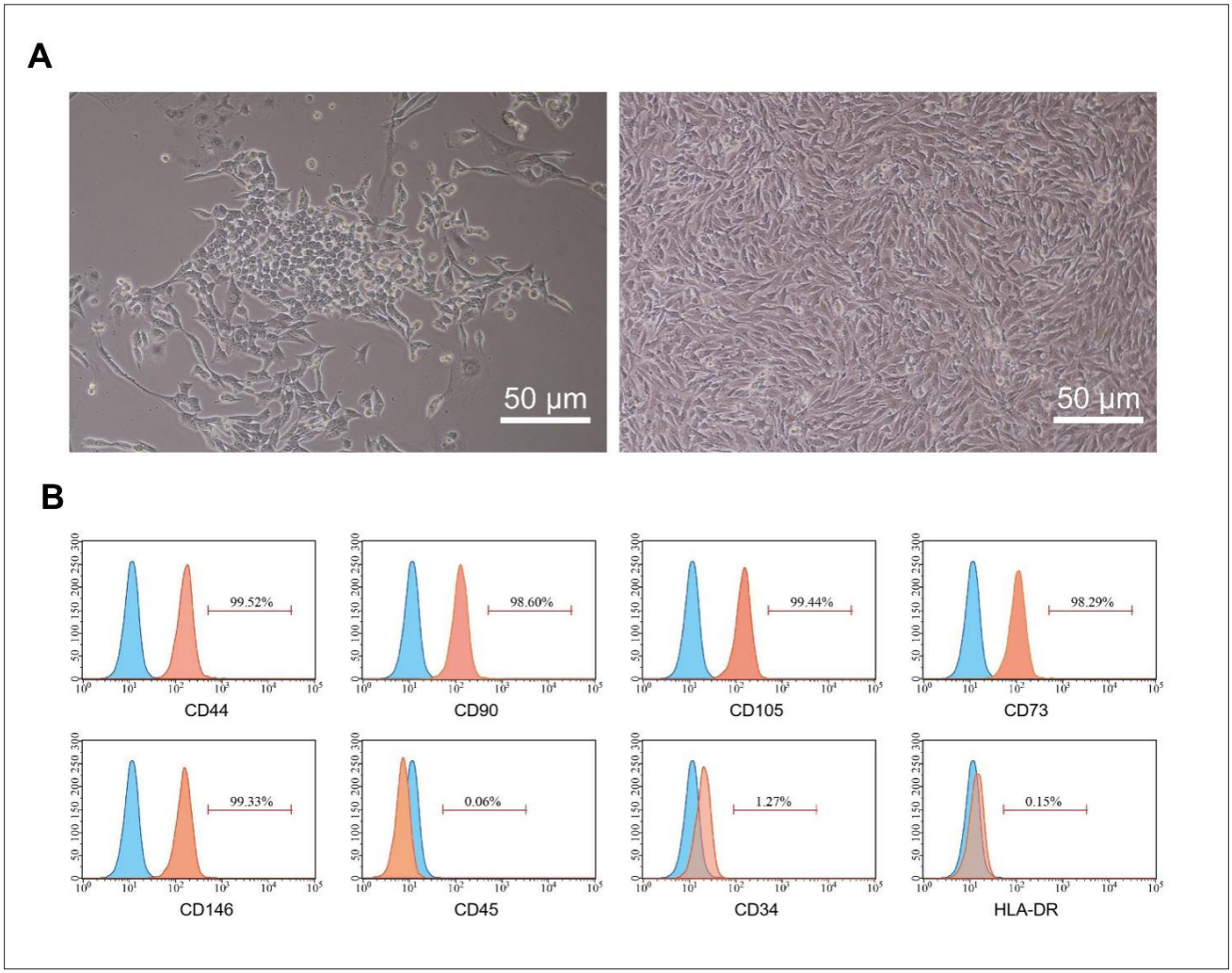

Figure S2. Isolation and characterization of BMSCs. (A) Morphology of BMSCs observed under a microscope at Day 6 of passage 0 (left) and Day 4 of passage 4 (right). (B) Flow cytometry analysis of positive surface marker expression in BMSCs. Magnifications: 100× (A, left); 200× (A, right). Scale bars: 50 μm (A). Abbreviation: BMSCs, bone marrow-derived mesenchymal stem cells; CD, cluster of differentiation; HLA-DR, human leukocyte antigen – DR isotype.

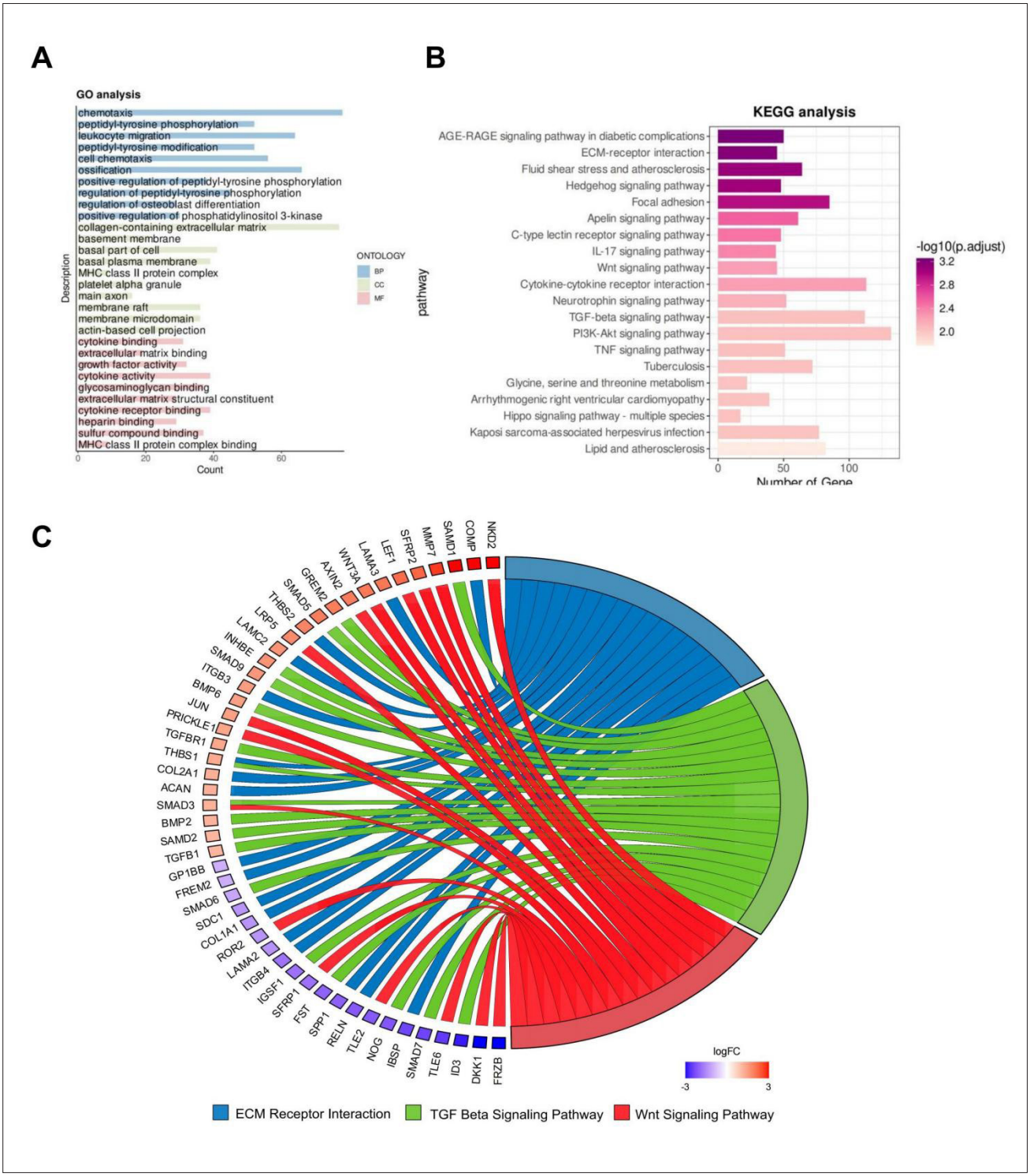

Figure S3. Functional enrichment analysis of DEGs. (A) GO functional enrichment analysis featuring the enrichment of DEGs in chondrogenic differentiation-related biological processes. (B) KEGG pathway enrichment analysis indicating significantly upregulated pathways after KGN treatment, including the Smad1/5/9 signaling pathway. (C) Gene enrichment in chondrogenic differentiation-related pathways, including TGF-β, Wnt, and ECM pathways. Abbreviations: BP, biological process; CC, cellular component; ECM, extracellular matrix; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; logFC, log fold change; MF, molecular function; TGF-β, transforming growth factor-beta.

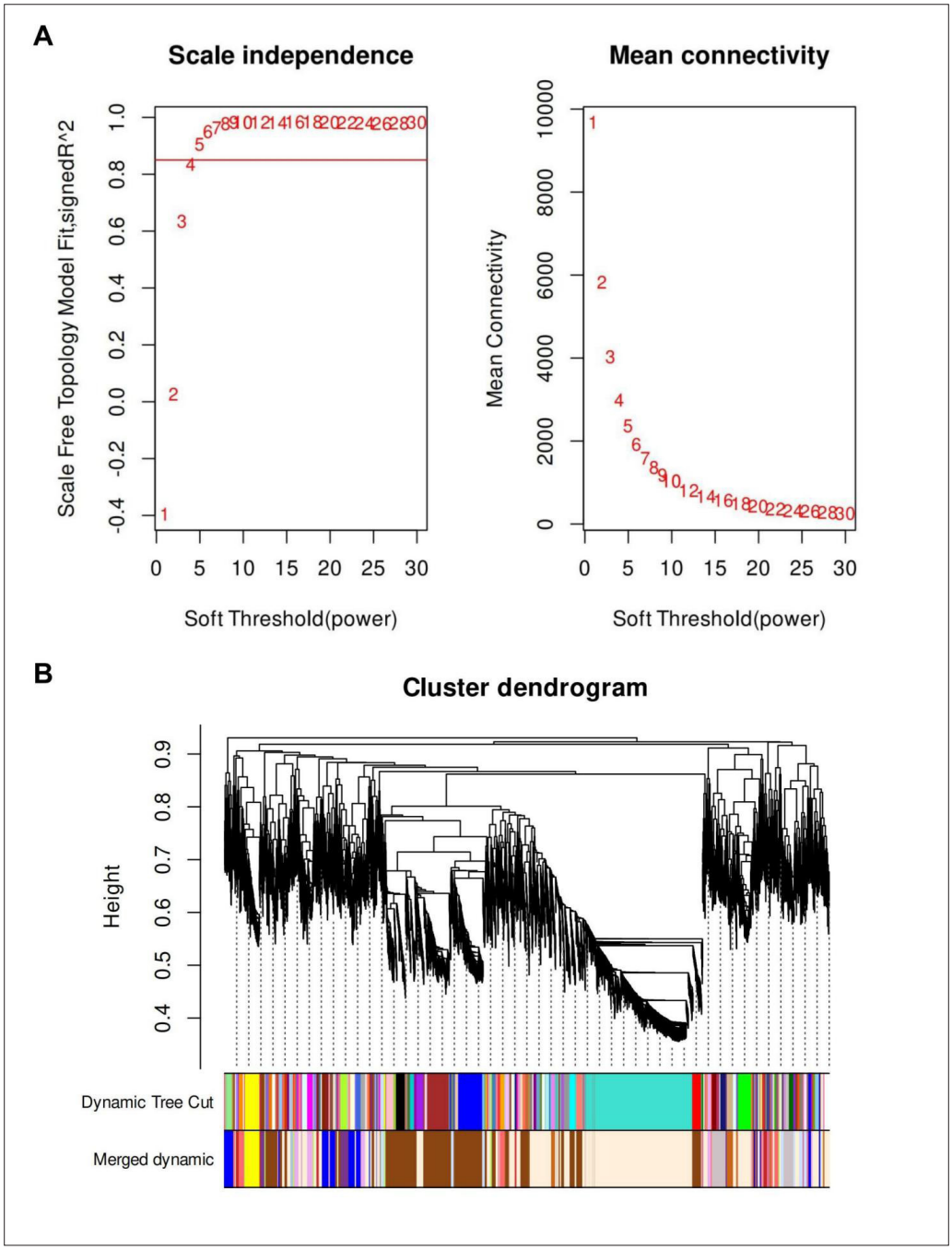

Figure S4. Weighted gene co-expression network analysis (WGCNA) of DEGs. (A) Analysis of scale-free fit index (left) and mean connectivity (right) for each soft-threshold power. (B) Cluster dendrogram of DEG modules. Abbreviation: DEGs, differentially expressed genes.

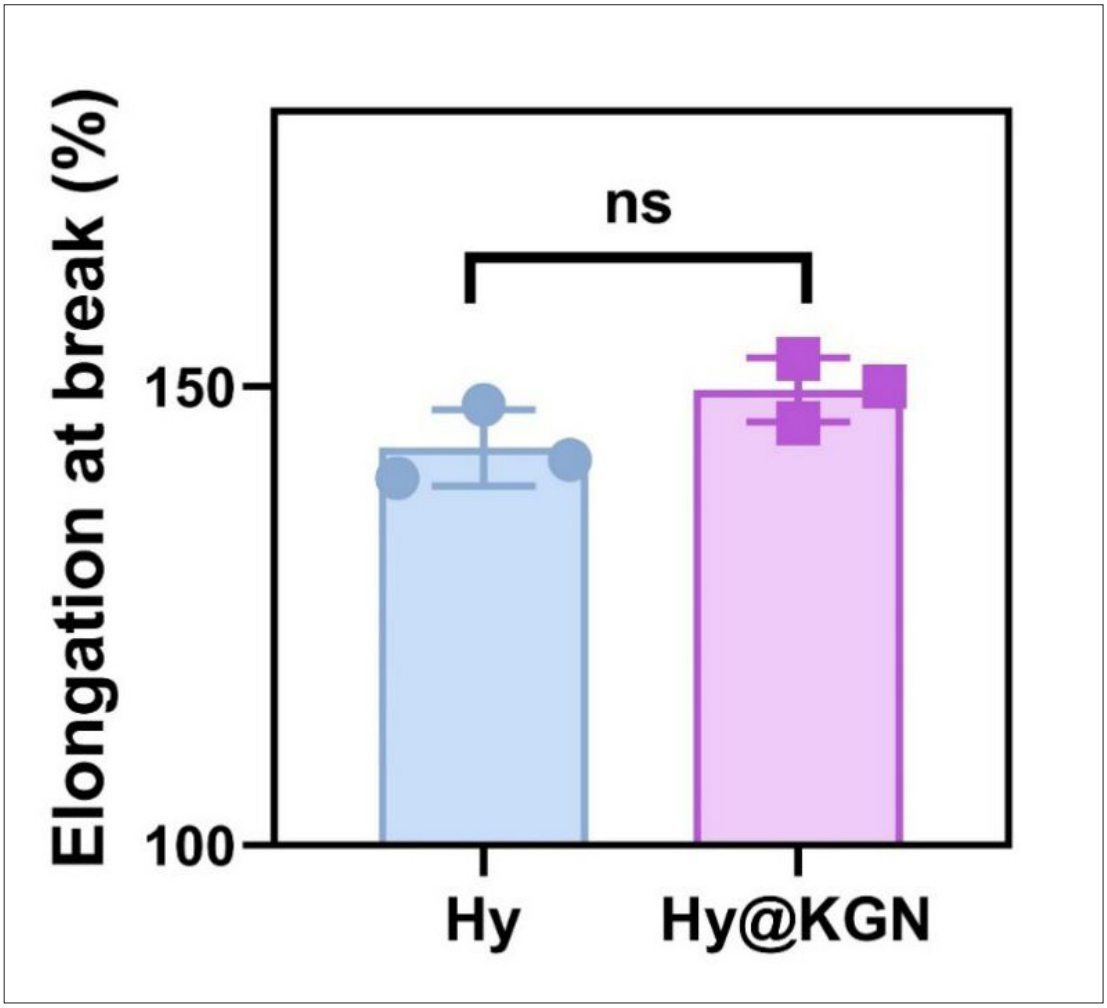

Figure S5. Elongation at break of Hy and Hy@KGN hydrogels (n = 3); ns, non-significant.

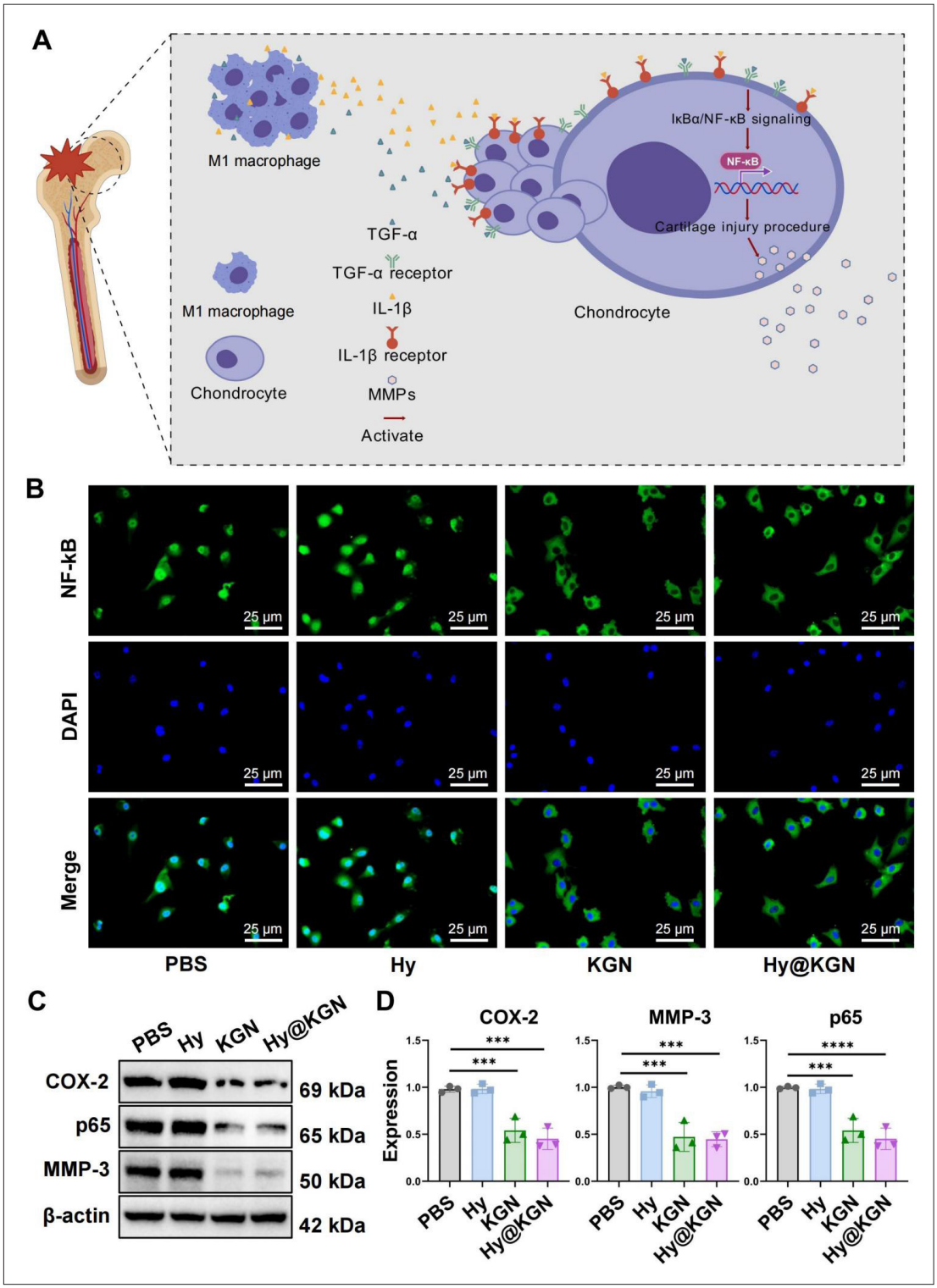

Figure S6. Effect of Hy@KGN hydrogel on inflammatory response in chondrocytes. (A) Schematic of the activation of the IκBα/NF-κB signaling pathway by inflammatory factors released from M1 macrophages. (B) Immunofluorescence staining to assess NF-κB nuclear translocation. (C) Western blot analysis of COX-2, MMP-3, and phosphorylated NF-κB (p65) expression levels in chondrocytes; (D) Quantitative analysis of COX-2, MMP-3, and phosphorylated NF-κB (p65) expression levels from (C). ***$p < 0.001$, ****$p < 0.0001$. All cell experiments were performed in triplicate. Scale bars: 25 μm (B). Abbreviations: COX-2, Cyclooxygenase-2; Hy@KGN, Kartogenin-loaded hydrogel; IκBα, inhibitor of nuclear factor kappa B alpha; KGN, Kartogenin; MMP-3, matrix metalloproteinase-3; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; p65, NF-κB subunit p65; PBS, phosphate-buffered saline.

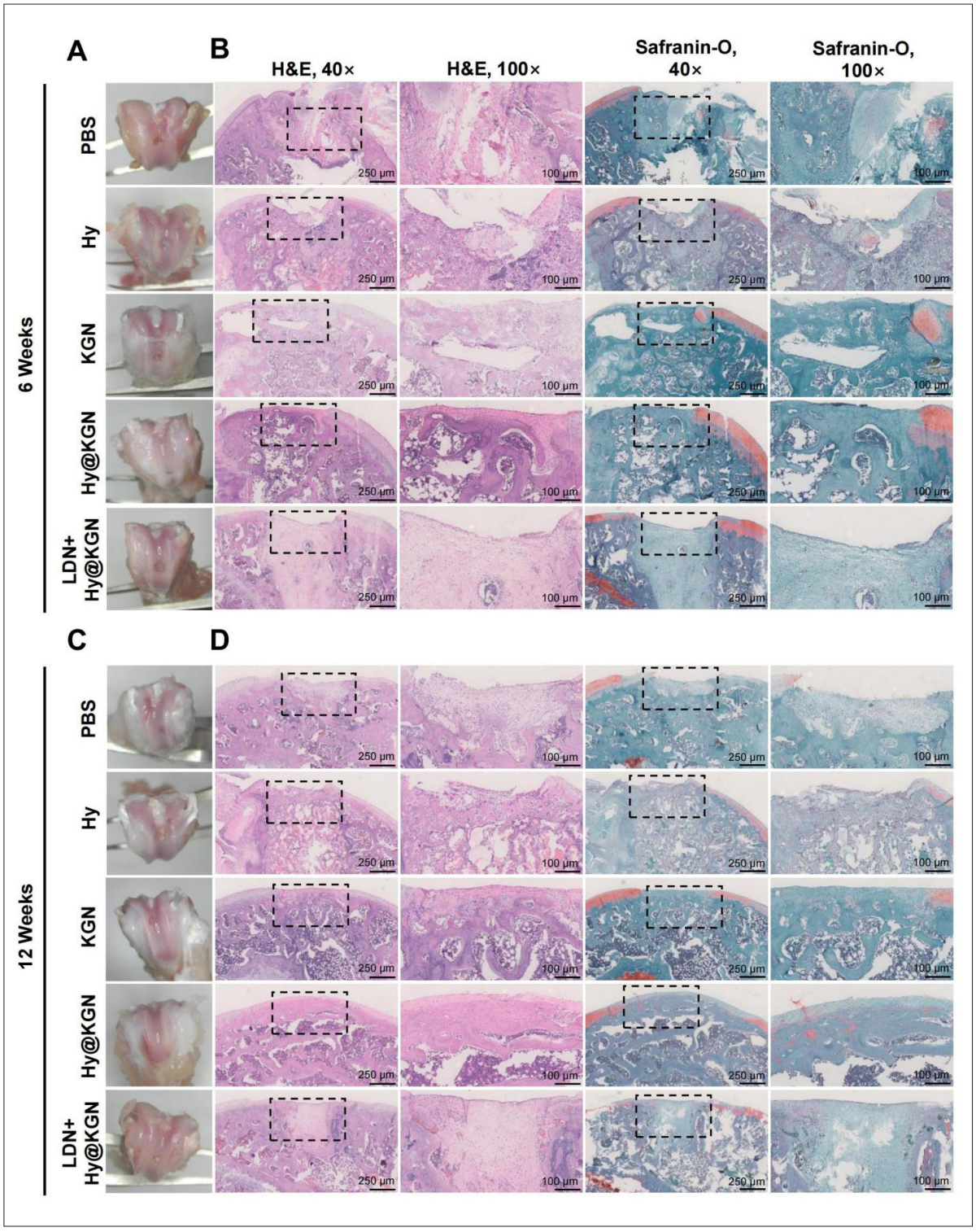

Figure S7. Morphological characteristics of cartilage tissue. (A) Macroscopic observation of cartilage defects at week 6 post-surgery. (B) H&E staining for cartilage structure (left) and SO/FG staining for new cartilage and bone tissue (right) at week 6. (C) Macroscopic observation of cartilage defects at week 12 post-surgery. (D) H&E staining for cartilage structure (left) and SO/FG staining for new cartilage and bone tissue (right) at week 12. Scale bars: 2 mm (A and C); 250/100 μm (B and D). Abbreviations: H&E, Hematoxylin and eosin; Hy@KGN, kartogenin-loaded hydrogel; PBS, phosphate-buffered saline; SO/FG, safranin O/fast green.…

Table S1. Hydrogel formulation

Hydrogel	Composition	Description
Hy	HEMA 0.5 g; PEGDA 0.5 g; APS 0.05 g; TEMED 0.05 mL	Base hydrogel with good biocompatibility and mechanical properties; no KGN loading
Hy@KGN	HEMA 0.5 g; PEGDA 0.5 g; APS 0.05 g; TEMED 0.05 mL; KGN: 0.1 g (dissolved in 1 mL ethanol)	KGN-loaded hydrogel designed to promote chondrogenic differentiation of BMSCs and cartilage repair

Abbreviations: APS, ammonium persulfate; BMSCs, bone marrow stromal cells; Hy, hydrogel without kartogenin; Hy@KGN, kartogenin-loaded hydrogel; HEMA, hydroxyethyl methacrylate; KGN, Kartogenin; PEGDA, poly(ethylene glycol) diacrylate; TEMED, N,N,N′,N′-tetramethylethylenediamine.

Table S2. RT-qPCR primer sequences (rat)

Gene ID	Primer sequence (5'–3')
Col2a1-F	ATCGCCACGGTCCTACAATG
Col2a1-R	CATCGCAGAGGACATTCCCA
Acan-F	GGGACCTGTGTGAGATCGAC
Acan-R	GGTCGGGAAAGTGGCGATAA
Sox9-F	GCAAACACGTTGCAAATGGC
Sox9-R	AACTCTGAAGGAGCCAAGCC
Prg4-F	TTCGAGTTGCTGCTTTGCAC
Prg4-R	GATTTGATGGTGCGAGACGC
Actb-F	CCCGCGAGTACAACCTTCTT
Actb-R	CGCAGCGATATCGTCATCCA

Note: F: Forward; R: Reverse. Abbreviation: RT-qPCR, reverse transcription quantitative polymerase chain reaction.