Supplementary Information (SI) for Journal of Materials Chemistry B.

Double Network Hydrogels Encapsulating Genetically Modified Dedifferentiated Chondrocytes for Auricular Cartilage Regeneration

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Supporting Figures

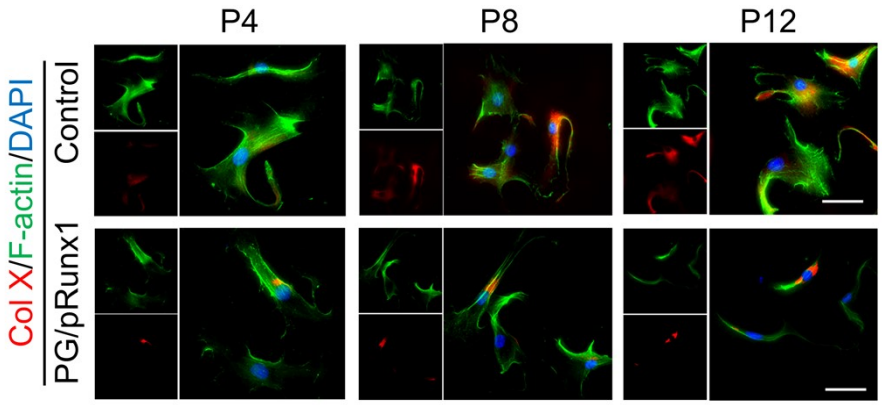

Fig. S1. Immunofluorescence staining of hypertrophic chondrocyte markers (Col X) was performed in chondrocytes at different passage numbers with or without PG/pRunx1 nanoparticles treatment. Scale

bars=50 μm.

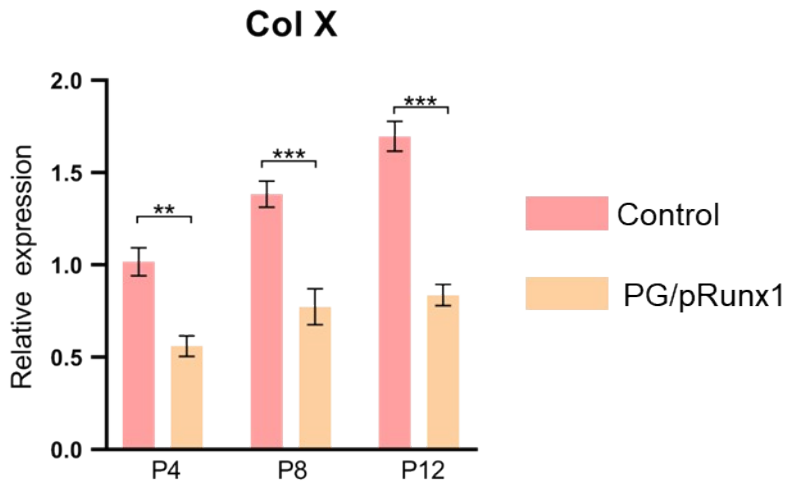

Fig. S2. RT-qPCR detection of hypertrophic chondrocyte markers (Col X) in chondrocytes at different passage numbers with or without PG/pRunx1 nanoparticles treatment.

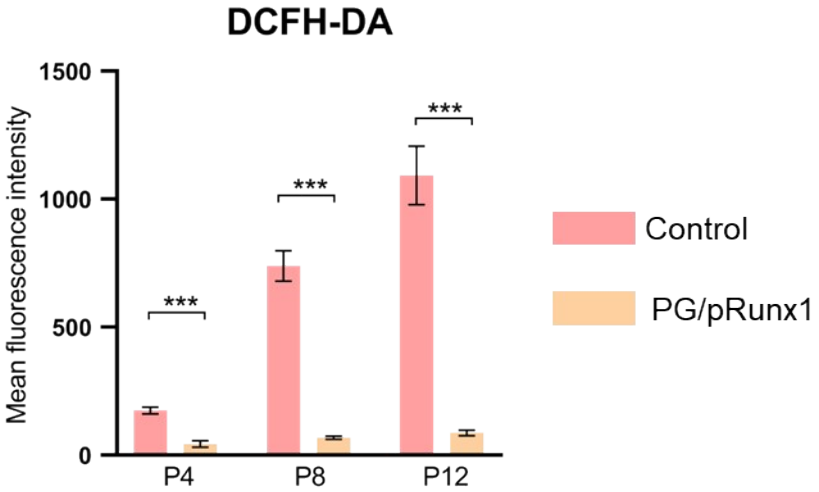

Fig. S3. Quantitative analysis of mean DCF fluorescence intensity in chondrocytes at different passage numbers with or without PG/pRunx1 nanoparticles treatment.

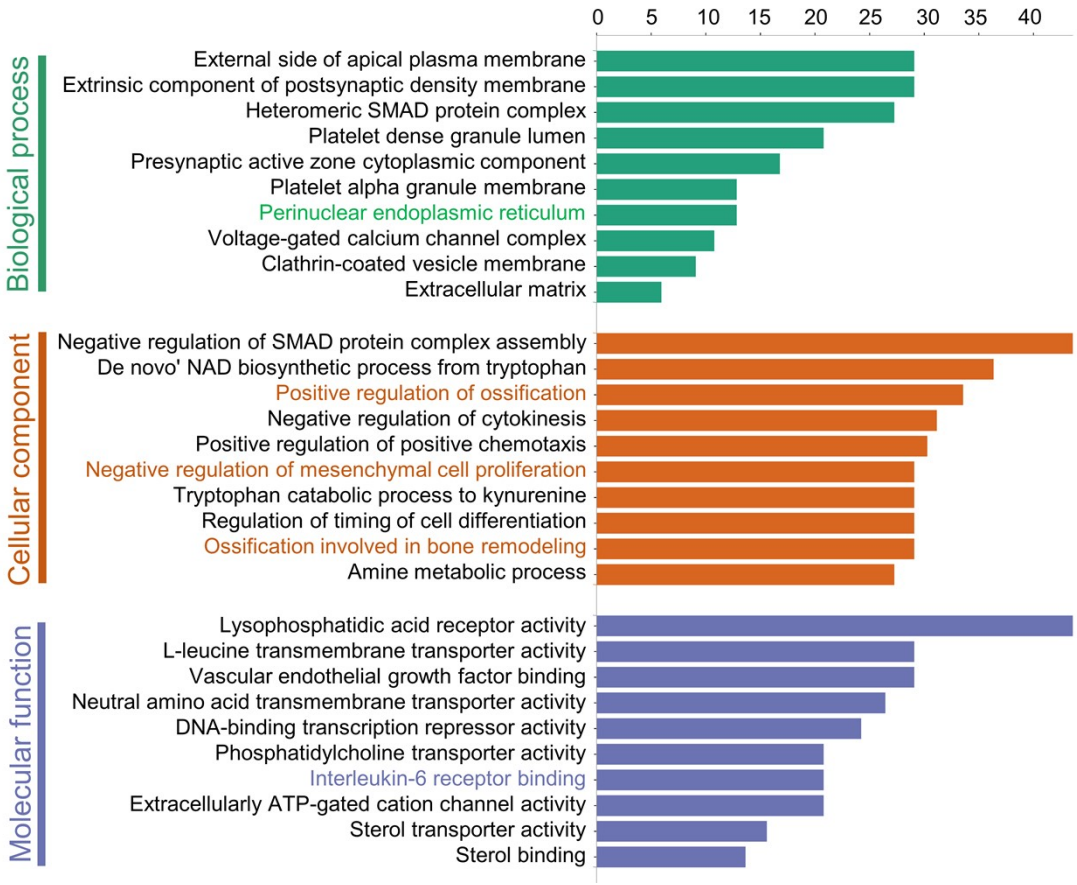

Fig. S4. The top 30 GO terms of the downregulated genes related to ER stress and cartilage regeneration in chondrocytes versus chondrocytes treated PG/pRunx1 nanoparticles.

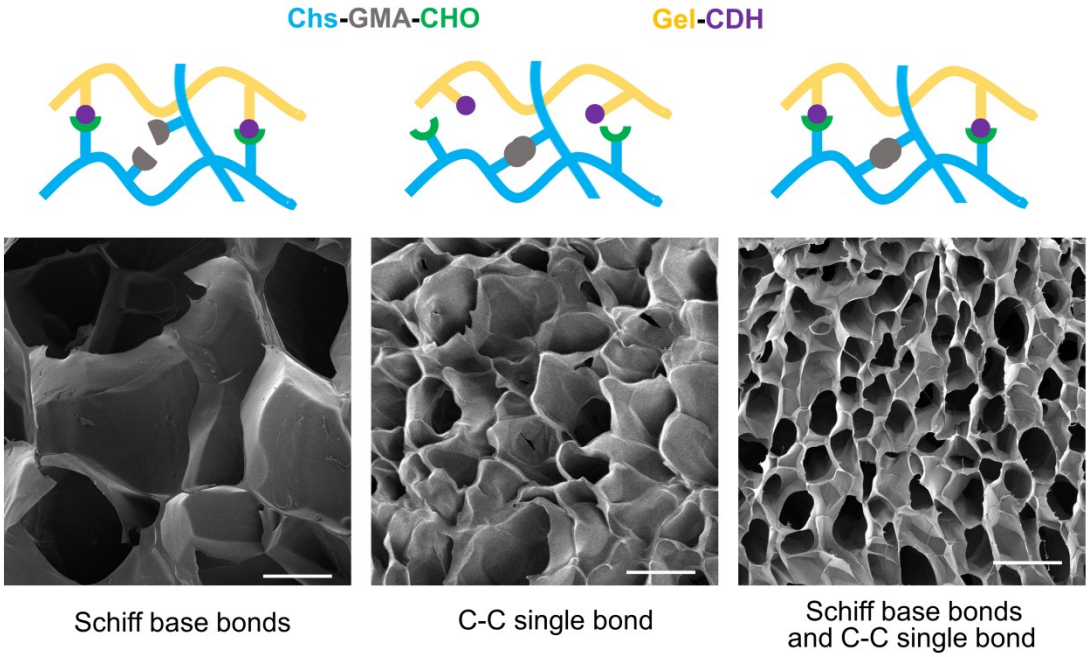

Fig. S5. The scanning electron microscopy (SEM) images displayed the different crosslinked hydrogels based on Chs-GMA-CHO and Gel-CDH. Scale bars=100 μm.

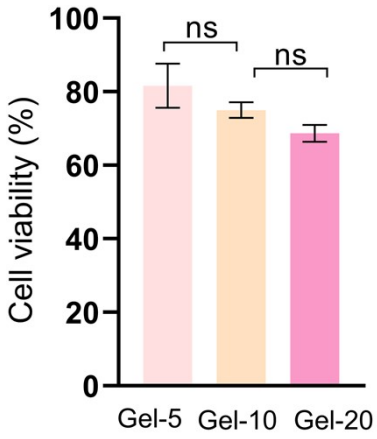

Fig. S6. The cell viability measurement of chondrocytes embedded in the different crosslinked hydrogels by CCK-8.

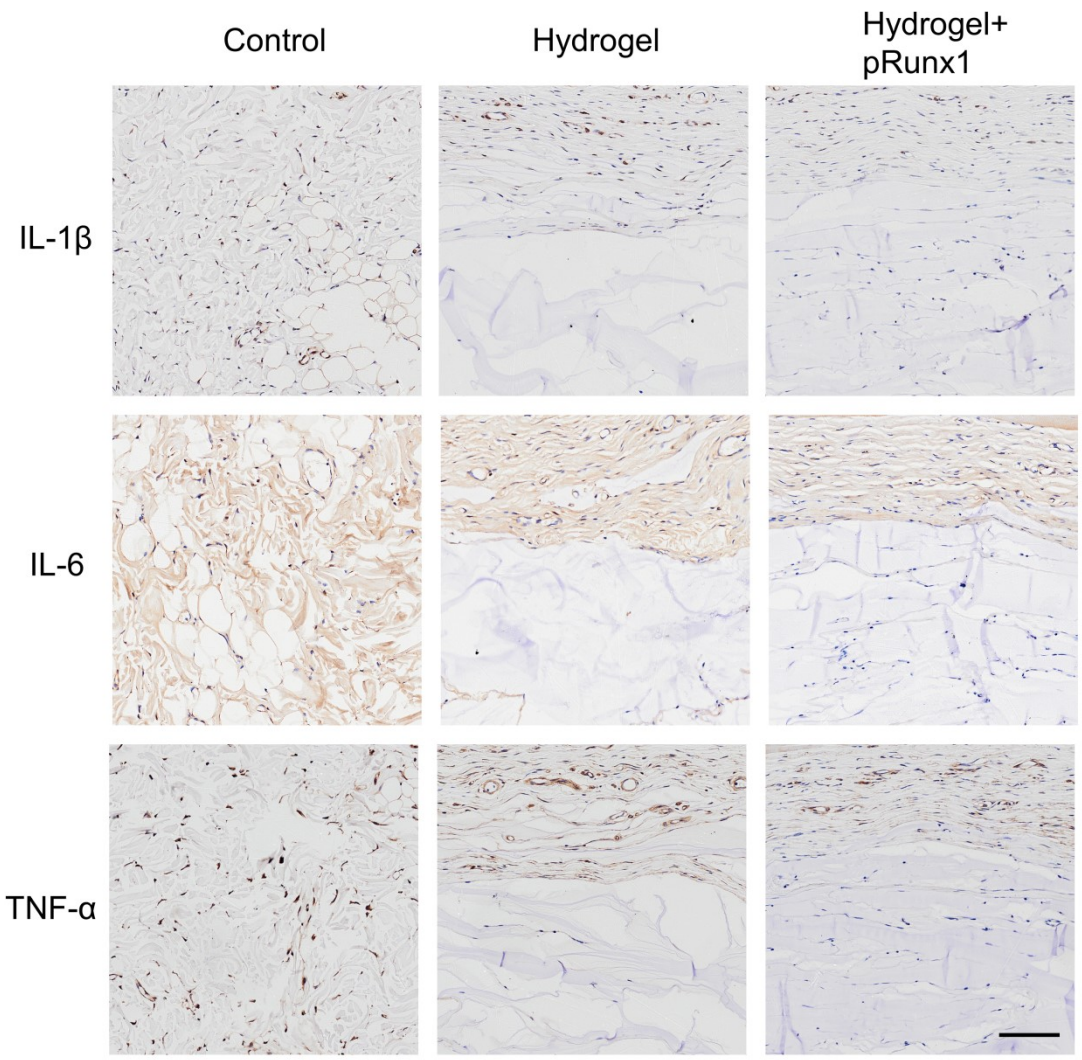

Fig. S7. Representative immunohistochemistry images of IL-1β, IL-6, and TNF-α in the hypodermis one week after hydrogels implantation. Scale bars= 100 μm.

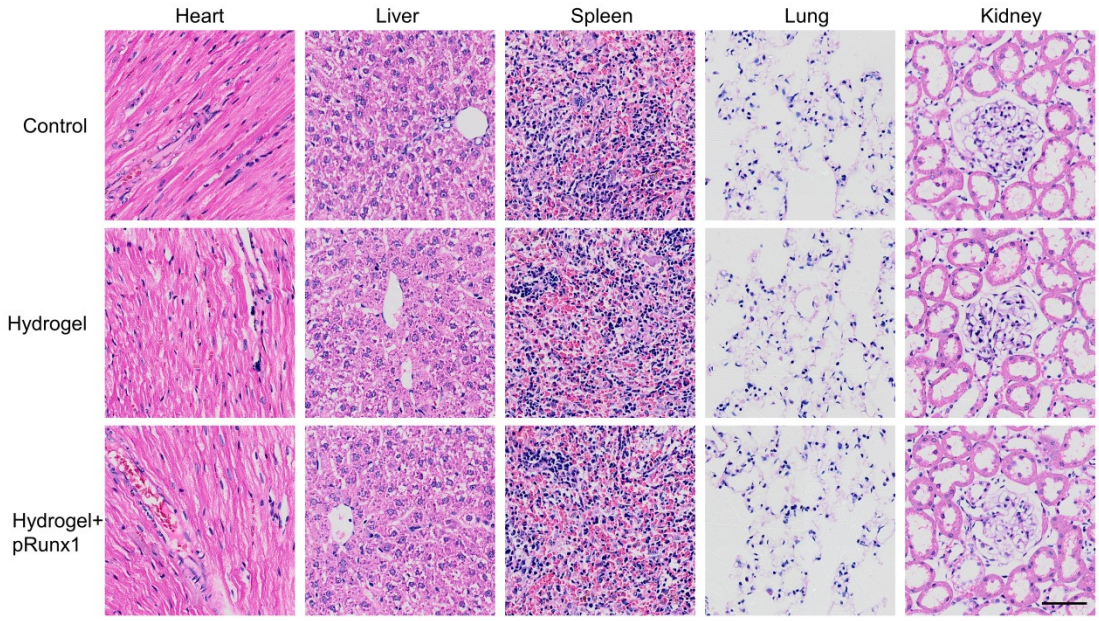

Fig. S8. Representative hematoxylin−eosin staining of the major organs (heart, liver, spleen, lung and kidney) of rats 1 weeks after subcutaneous implantation. Scale bars=50 μm.

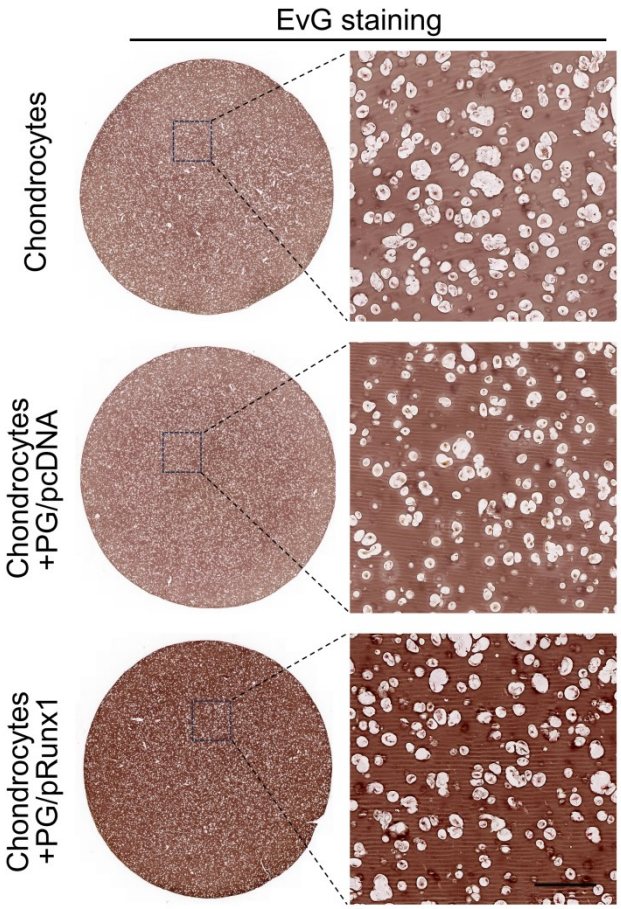

Fig. S9. The elastin in chondrocyte-encapsulated hydrogel disks were investigated with EvG staining, scale bars=200 μm.

Table S1. Primers for qRT-PCR.

Genes	Forward primer	Reverse primer
GAPDH	5-CGTCAAAGGTGGAGGAGTG-3	5-GAAGCTCACTGGCATGGC-3
Collagen 2A1	5-AGGTCACAGAGGTTATCCAG-3	5-GTCCGTCCTCTTTCACCAG-3
Aggrecan	5-TAAATACGGTGCTGCTGGC-3	5-TGGTGTGAGGACGTATGGC-3
SOX9	5-TCTGGAGACTTCTGAACGAGAGC-3	5-TGTAATCCGGGTGGTCCTTC-3
MMP13	5-CCTTCAAAGTTTGGTCCGATG-3	5-TCAAATGGGTAGAAGTCGCC-3
Collagen X	5-AGGAATGCCTGTGTCTGCTT-3	5-ACAGGCCTACCCAAACATGA-3
ATF4	5-ATGGATTTGAAGGAGTTCGACT-3	5-AGAGATCACAAGTGTCATCCAA-3
Runx1	5-GTGGTCCTACGATCAGTCCT-3	5-GTTCTGCAGAGAGGGTTGTC-3

Table S2. Composition of hydrogels.

Sample	Chs-GMA-CHO (mg)	Gel-CDH (mg)	PEO (mg)	LAP (mg)	PBS (mL)
Hydrogel-5 (Gel-5)	250	750	50	12.5	5
Hydrogel-10 (Gel-10)	500	750	50	12.5	5
Hydrogel-20 (Gel-20)	1000	750	50	12.5	5