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Dual-network DNA-Silk fibroin hydrogels with controllable surface rigidity for regulating chondrogenic differentiation

Ziyang Zhou[a,b,c,d,e,#], Peiran Song[a,b,c,#], Yan Wu[a,b,c,#], Miaomiao Wang[a,b,c,#], Congyi Shen[a,b,c,d,e], Zhixin Ma[a,b,c,d,e], Xiaoxiang Ren[a,b,c], Xiuhui Wang[a,b,c], Xiao chen[f], Yan Hu[f], Zuhao Li[a,b,c,f], Qin Zhang[a,b,c,*], Mengmeng Li[a,b,c,*], Zhen Geng[a,b,c,*], and Jiacan Su[a,b,c,f,*]

a Institute of Translational Medicine, Shanghai University, Shanghai, 200444, China.

b Organoid Research Center, Shanghai University, Shanghai, 200444, China.

c National Center for Translational Medicine (Shanghai) SHU Branch，Shanghai University, Shanghai, 200444, China.

d School of Medicine, Shanghai University, Shanghai, 200444, China.

e School of Life Sciences, Shanghai University, Shanghai, 200444, China.

f Department of Orthopedics, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 200092, China.

Table S1. DNA sequences information (The bold sequences are the sticky ends).

	ssDNA sequence (5' to 3')	Length (nt)
Y1	CCTGTCTGCCTAATGTGCGTCGTAAG**TAACTGGACACTT**	39
Y2	CTTACGACGCACAAGGAGATCATGAG**TAACTGGACACTT**	39
Y3	CTCATGATCTCCTTTAGGCAGACAGG**TAACTGGACACTT**	39
L1	CTACGGTGAATGGAATTCTCATGCGAATAGA**AAGTGTCCAGTT A**	44
L2	TCTATTCGCATGAGAATTCCATTCACCGTAG**AAGTGTCCAGTTA**	44

Table S2. Primer sequences used for quantitative RT-PCR

Target gene	Forward primer	Reverse primer
GAPDH	GAAGAAGGTGGTGAAGCAGG	CACTGTTGAAGTCGCAGGAG
Sox 9	GCGGAGGAAGTCGGTGAAGAA T	AAGATGGCGTTGGGCGAGAT
ACAN	GGAGGAGCAGGAGTTTGTCAA	TGTCCATCCGACCAGCGAAA
COL II	CACGCTCAAGTCCCTCAACA	TCTATCCAGTAGTCACCGCTC T

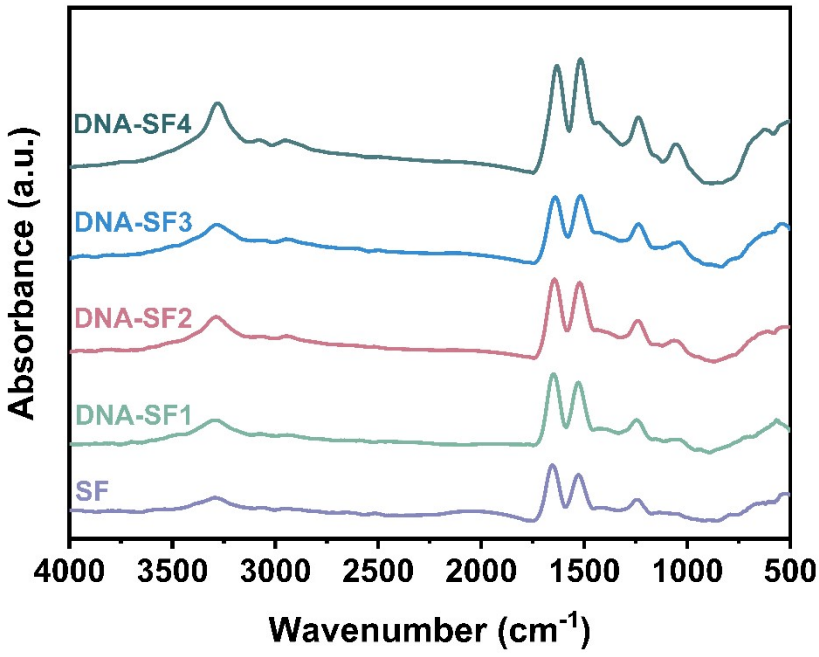

Figure S1. Full FTIR spectra of SF hydrogels and DNA-SF hydrogels.

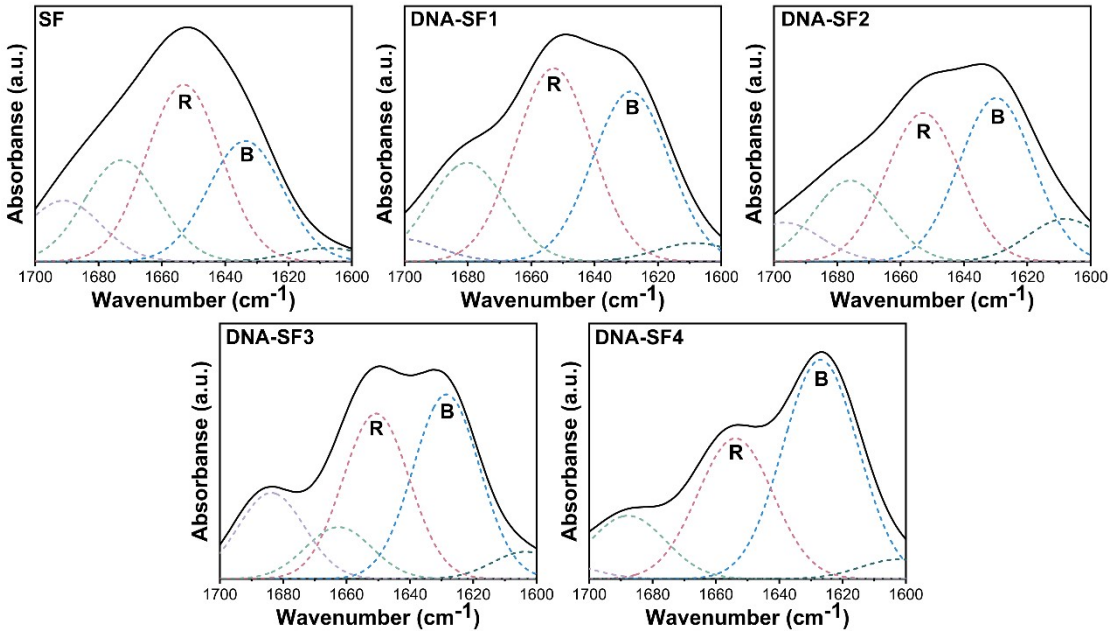

Figure S2. Absorbance spectra of SF hydrogels and DNA-SF hydrogels deduced after Fourier self-deconvolution (random coil marked as R and β-sheet marked as B).

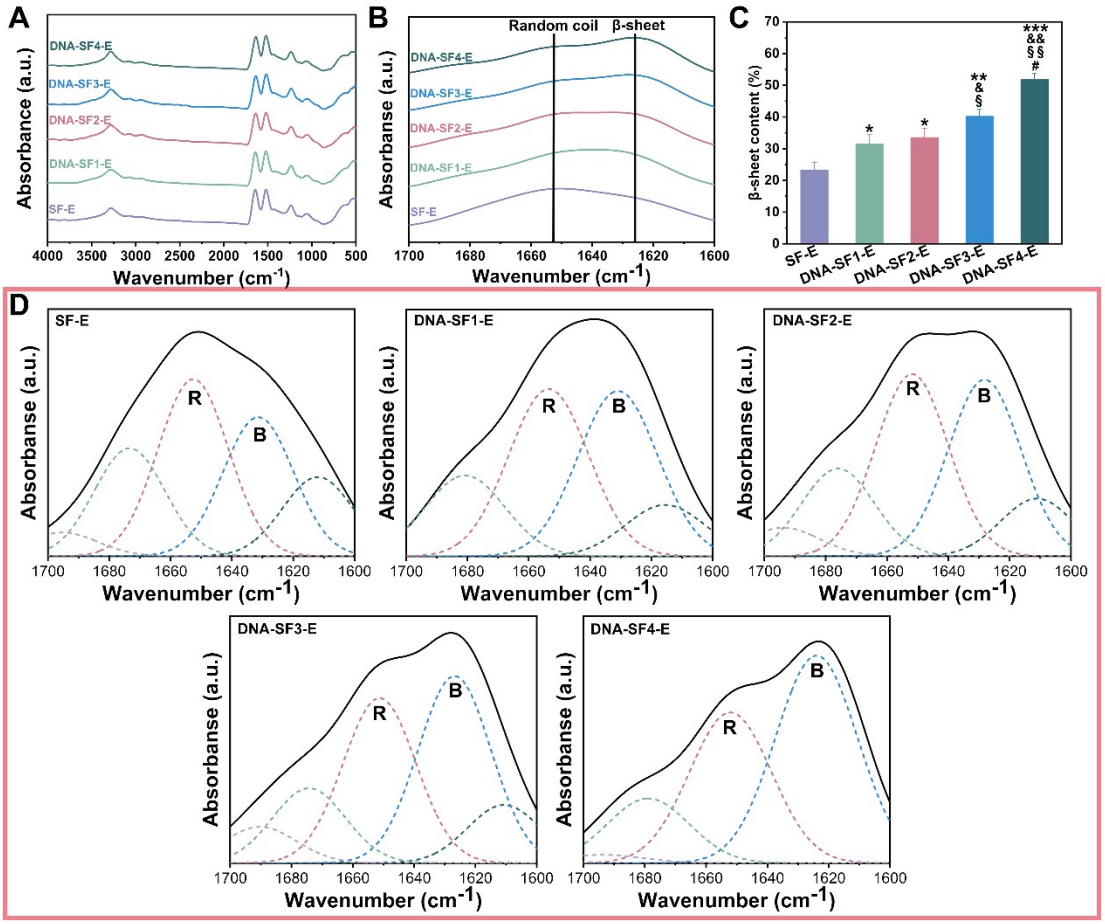

Figure S3. (A) Full FTIR spectra of SF-E hydrogels and DNA-SF-E hydrogels. (B) FTIR analysis of SF-E hydrogels and DNA-SF-E hydrogels (1600-1700 cm^{-1}). (C) β-sheet content of SF hydrogels and dual-network DNA-SF hydrogels. (D) Absorbance spectra of SF-E hydrogels and DNA-SF-E hydrogels deduced after Fourier self-deconvolution (random coil marked as R and β-sheet marked as B). (*: compared to value at SF-E groups, &: compared to value at DNA-SF1-E groups, §: compared to value at DNA-SF2-E groups, #: compared to value at DNA-SF3-E groups.)

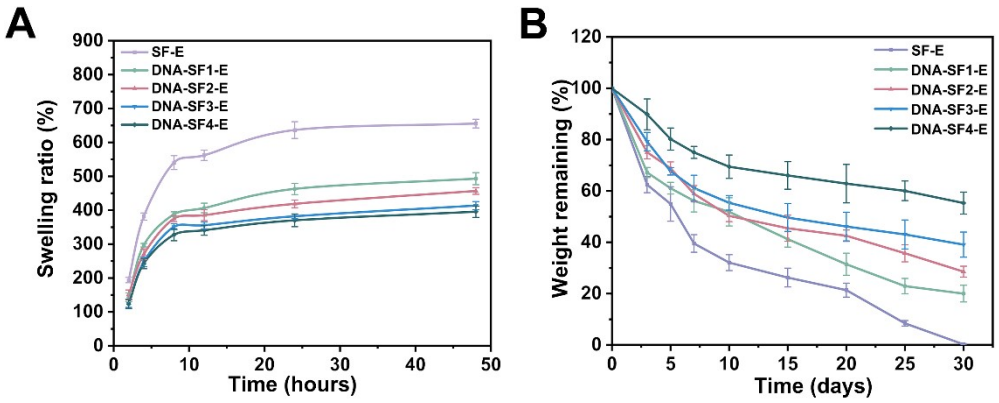

Figure S4. (A) The swelling ratio of SF-E hydrogels and DNA-SF-E hydrogels in PBS at 37 ℃. (B) *In vitro* degradation curves of SF-E hydrogels and DNA-SF-E hydrogels in protease XIV solution at 37 ℃.

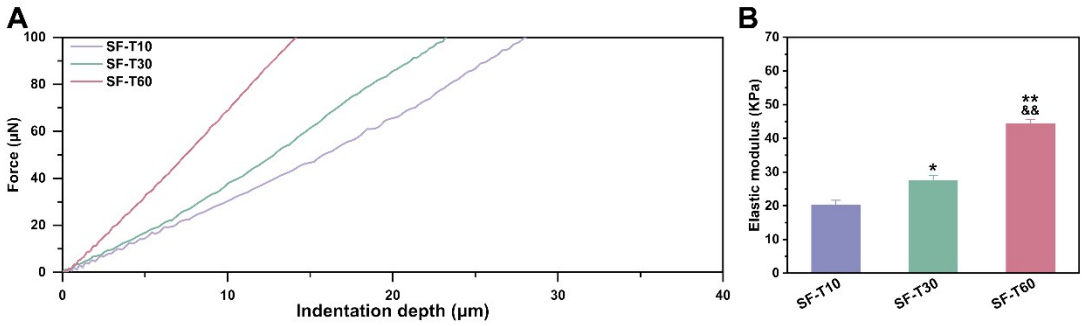

Figure S5. Mechanical properties of the SF-T10, SF-T30 and SF-T60 hydrogels (treat with ethanol for 10, 30, and 60 seconds respectively). (A) Stress-strain curves of SF hydrogels tested by nanoindenter. (B) Elastic modulus of SF hydrogels. (*: compared to value at SF-T30 group, &: compared to value at SF-T30 group.)

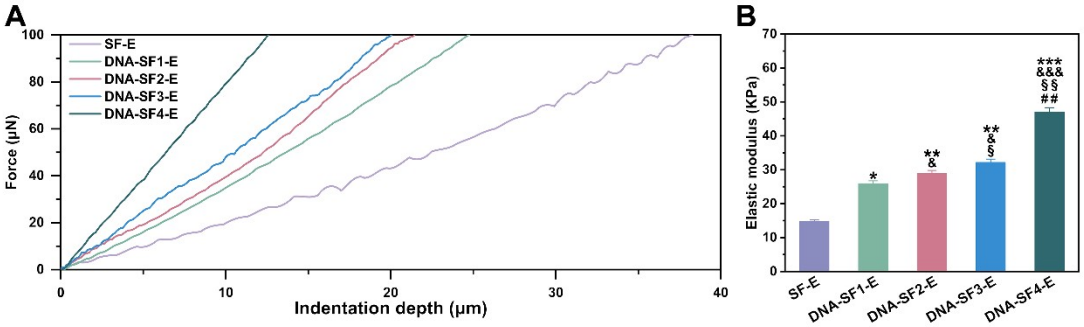

Figure S6. Mechanical properties of the SF-E hydrogels and DNA-SF-E hydrogels. (A) Stress-strain curves of SF hydrogels tested by nanoindenter. (B) Elastic modulus of SF hydrogels. (*: compared to value at SF-E group, &: compared to value at DNA-SF1-E group, §: compared to value at DNA-SF2-E group, #: compared to value at DNA-SF3-E group.)

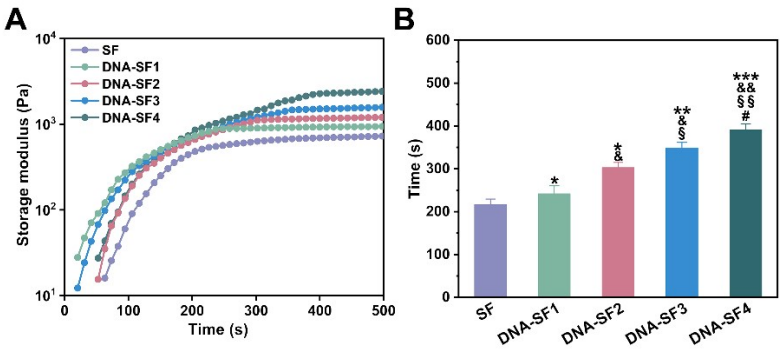

Figure S7. (A) The time scan of SF hydrogels and DNA-SF hydrogels. (B) The time required for the hydrogels to reach a stable state. (*: compared to value at SF-E group, &: compared to value at DNA-SF1-E group, §: compared to value at DNA-SF2-E group, #: compared to value at DNA-SF3-E group.)

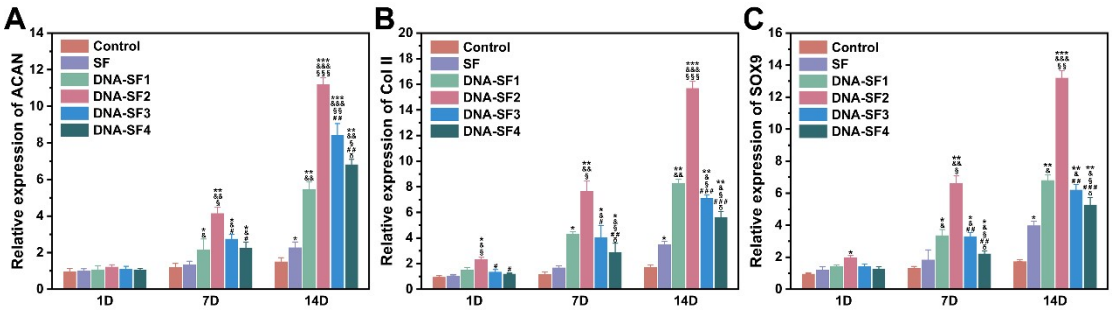

Figure S8. qRT-PCR results of mRNA expression of (A) ACAN, (B) Sox9 and (C) collagen II in BMSCs cultured on SF hydrogels and DNA-SF hydrogels for 1, 7 and 14 days. (*: compared to

value at Control groups, &: compared to value at SF groups, §: compared to value at DNA-SF1 groups, #: compared to value at DNA-SF2 groups, δ: compared to value at DNA-SF3 groups.)

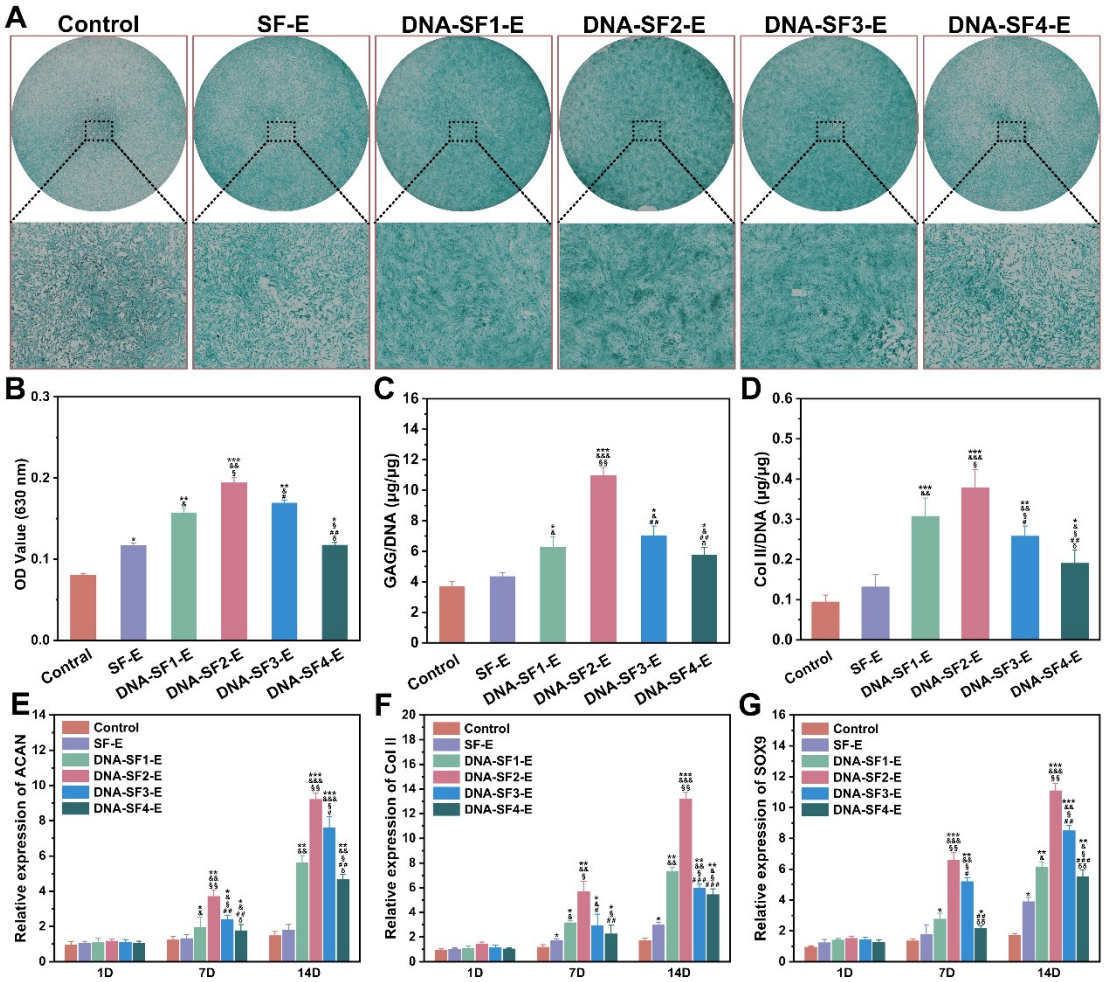

Figure S9. The effect of hydrogels-E on BMSCs chondrogenic differentiation. (A) Alcian staining of the BMSCs cultured on the SF-E hydrogels and DNA-SF-E hydrogels for 7 days (scale bars 200 μm). (B) Quantification of alcian blue staining. (C and D) Relative production of GAG and collagen II for BMSCs cultured on the SF-E hydrogels and DNA-SF-E hydrogels at 7 days. (E-G) qRT-PCR results of mRNA expression of ACAN, Col II and Sox9 in BMSCs cultured on SF-E hydrogels and DNA-SF-E hydrogels for 1, 7 and 14 days. (*: compared to value at Control groups, &: compared to value at SF-E groups, §: compared to value at DNA-SF1-E groups, #: compared to value at DNA-SF2-E groups, δ: compared to value at DNA-SF3-E groups.)

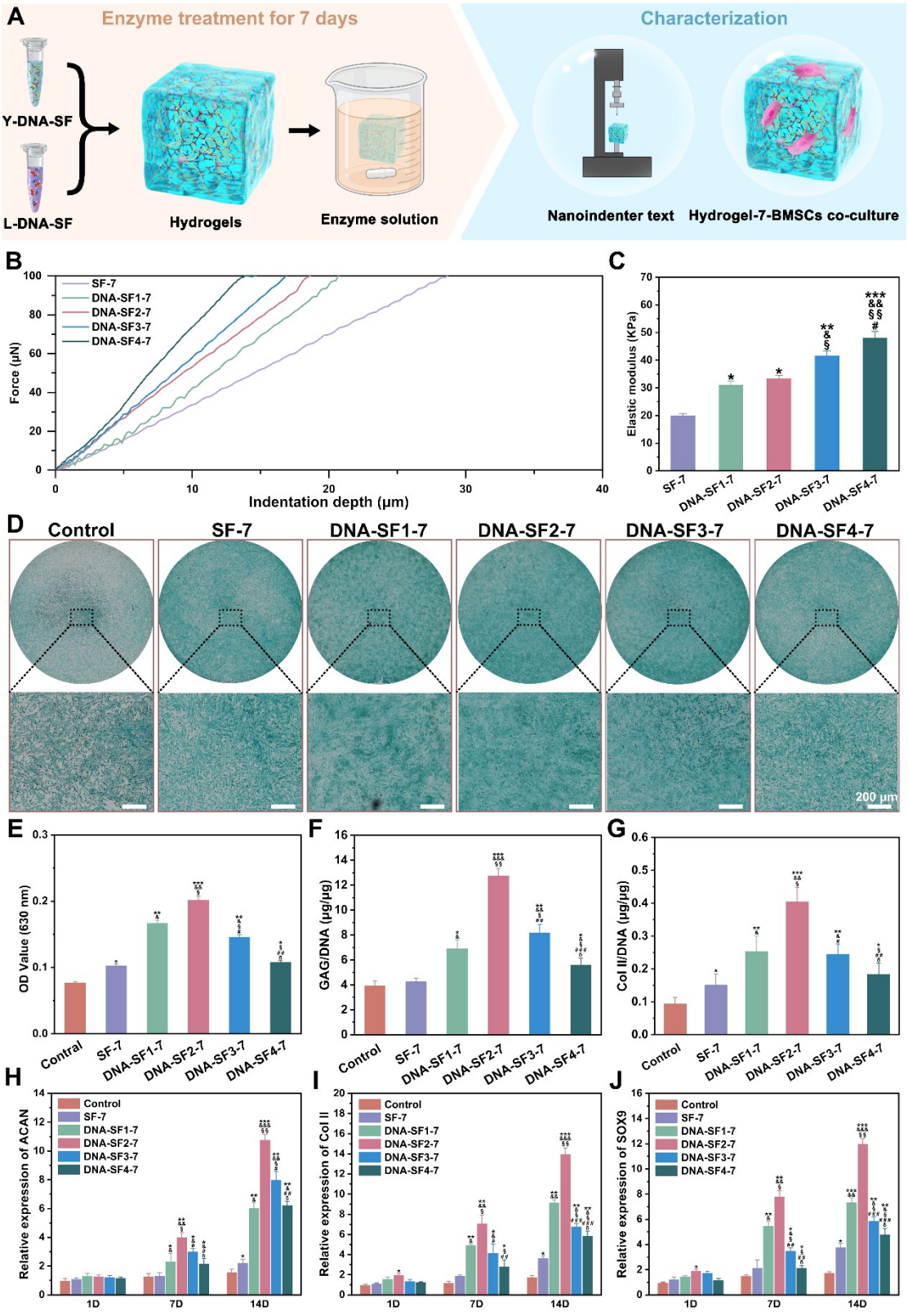

Figure S10. Dynamic analysis of SF hydrogels and DNA-SF hydrogels (D 7). (A) Schematic diagram of dynamic analysis experiment (D 7). (B) Stress-strain curves of SF-7 hydrogels and DNA-SF-7 hydrogels tested by nanoindenter. (C) Elastic modulus of SF-7 hydrogels and DNA-SF-7 hydrogels. (D) Alcian staining of the BMSCs cultured on the SF-7 hydrogels and DNA-SF-7

hydrogels for 7 days (scale bars 200 μm). (B) Quantification of alcian blue staining. (F and G) Relative production of GAG and collagen II for BMSCs cultured on the SF-7 hydrogels and DNA-SF-7 hydrogels at 7 days. (H-J) qRT-PCR results of mRNA expression of ACAN, Col II and Sox9 in BMSCs cultured on SF-7 hydrogels and DNA-SF-7 hydrogels for 1, 7 and 14 days. (*: compared to value at Control groups, &: compared to value at SF-7 groups, §: compared to value at DNA-SF1-7 groups, #: compared to value at DNA-SF2-7 groups, δ: compared to value at DNA-SF3-7 groups.)

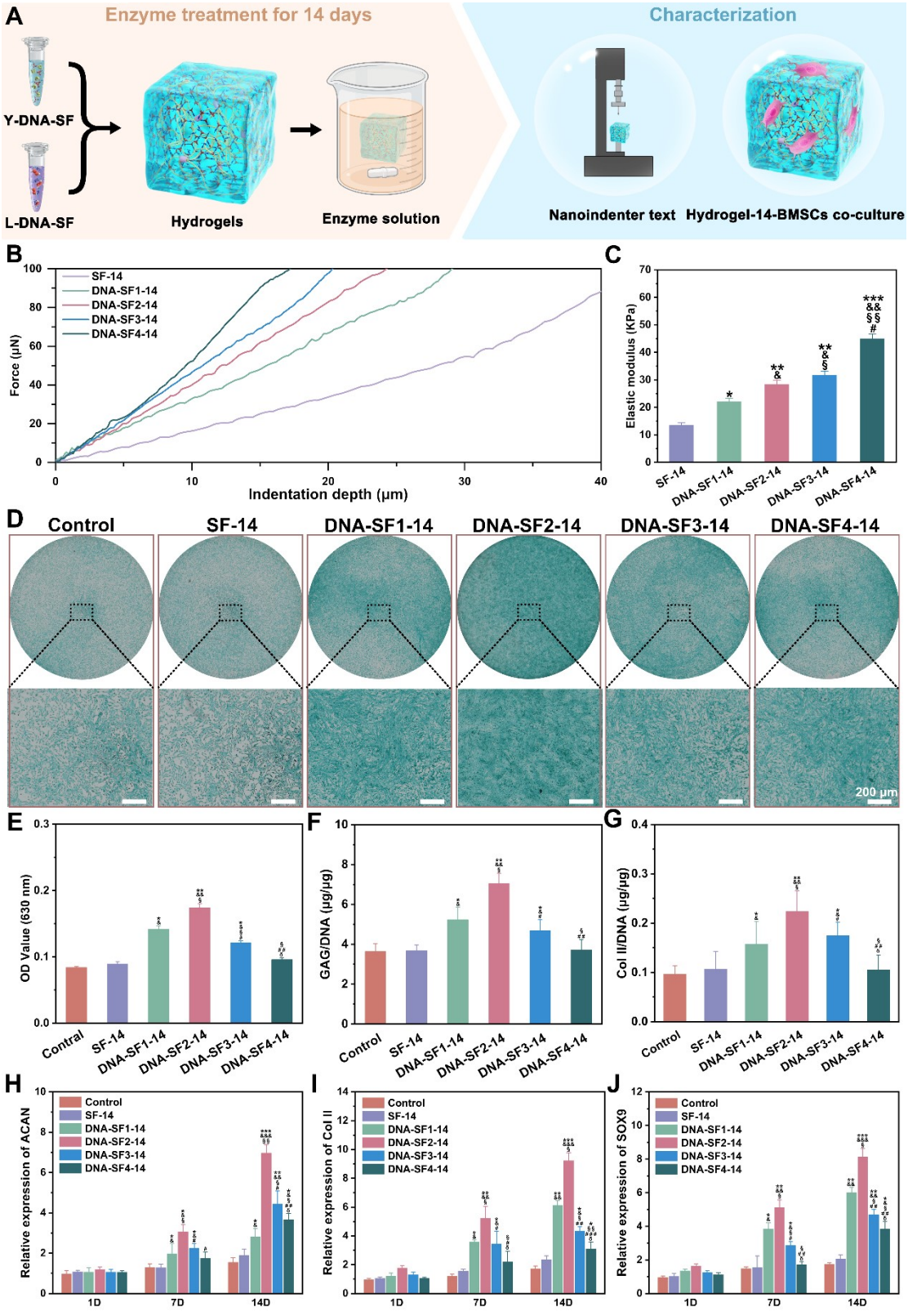

Figure S11. Dynamic analysis of SF hydrogels and DNA-SF hydrogels (D 14). (A) Schematic diagram of dynamic analysis experiment (D 14). (B) Stress-strain curves of SF-14 hydrogels and DNA-SF-14 hydrogels tested by nanoindenter. (C) Elastic modulus of SF-14 hydrogels and DNA-SF-14 hydrogels. (D) Alcian staining of the BMSCs cultured on the SF-14 hydrogels and DNA-SF-

14 hydrogels for 7 days (scale bars 200 μm). (B) Quantification of alcian blue staining. (F and G) Relative production of GAG and collagen II for BMSCs cultured on the SF-14 hydrogels and DNA-SF-14 hydrogels at 7 days. (H-J) qRT-PCR results of mRNA expression of ACAN, Col II and Sox9 in BMSCs cultured on SF-14 hydrogels and DNA-SF-14 hydrogels for 1, 7 and 14 days. (*: compared to value at Control groups, &: compared to value at SF-14 groups, §: compared to value at DNA-SF1-14 groups, #: compared to value at DNA-SF2-14 groups, δ: compared to value at DNA-SF3-14 groups.)

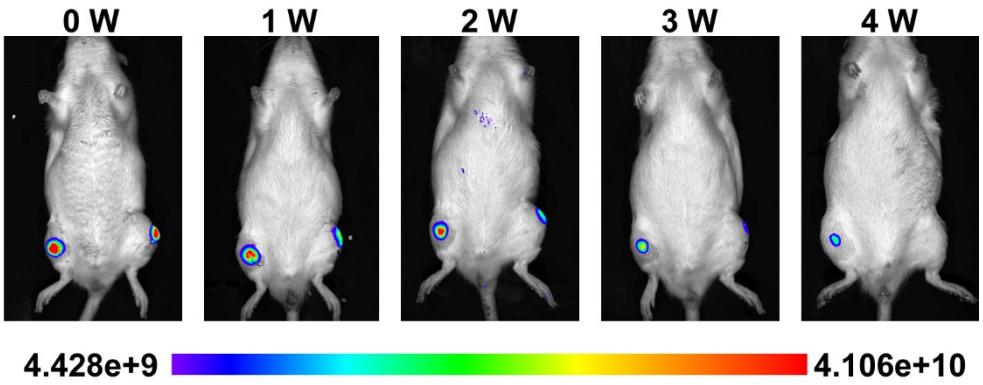

Figure S12. *In vivo* degradation behavior analysis of SF hydrogel (left hind legs) and DNA-SF hydrogels (left hind legs).

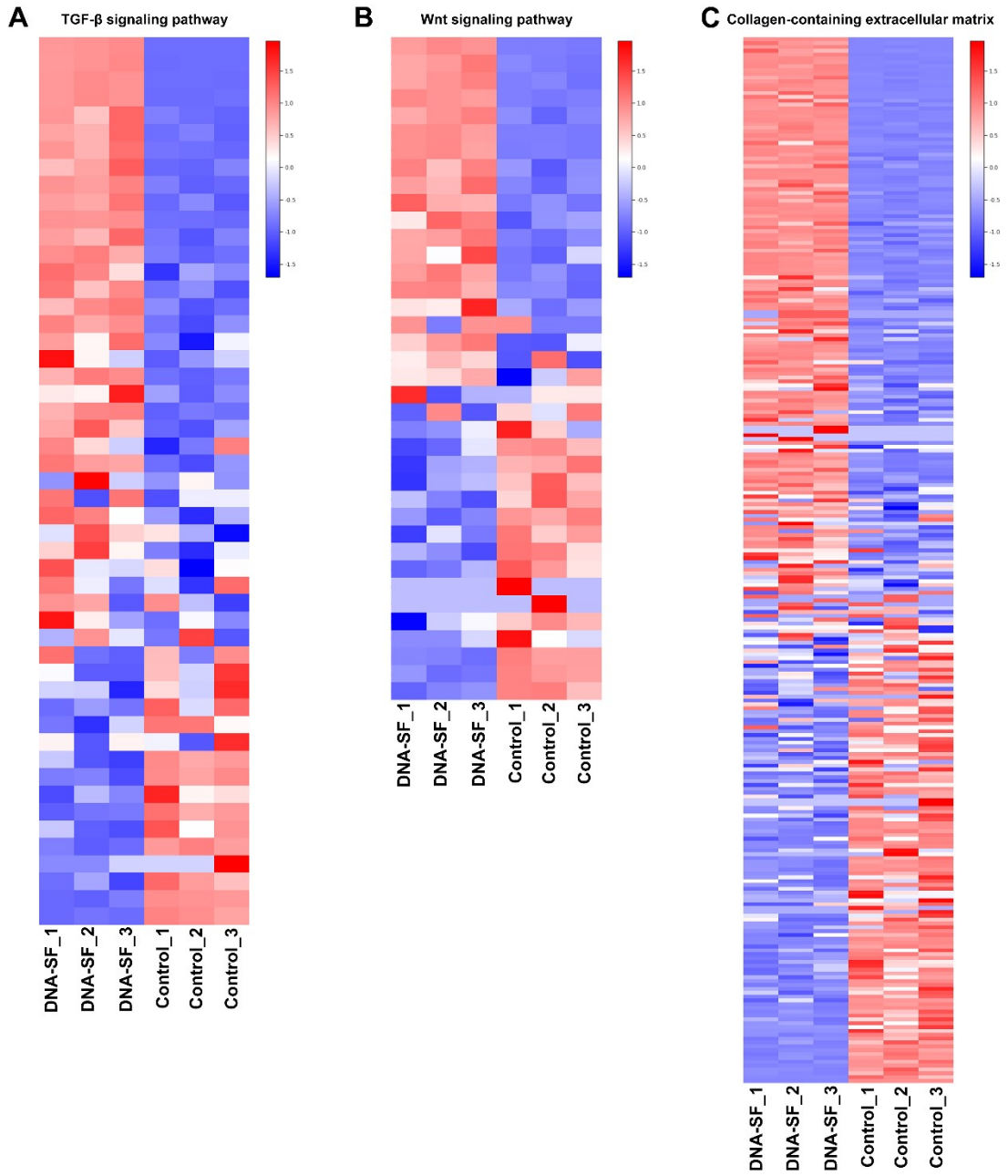

Figure S13. Heatmap of (A) TGF-β signaling pathway, (B) Wnt signaling pathway and (C) Collagen-containing extracellular matrix.

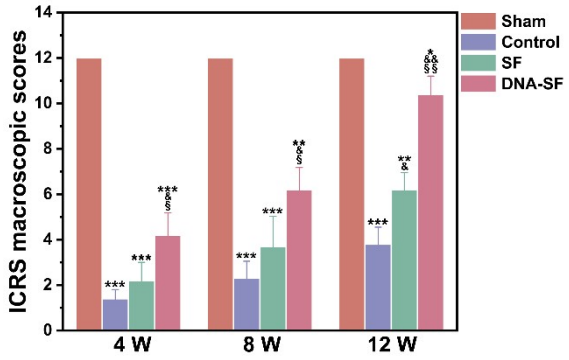

Figure S14. ICRS macroscopic score grading of the cartilage defect at 4, 8 and 12 weeks after treatment. (*: compared to value at Sham groups, &: compared to value at Control groups, §: compared to value at SF groups.)

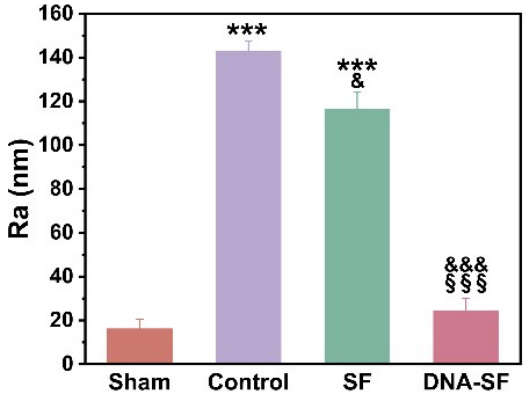

Figure S15. Quantitative analysis of cartilage surface roughness. (*: compared to value at Sham groups, &: compared to value at Control groups, §: compared to value at SF groups.)

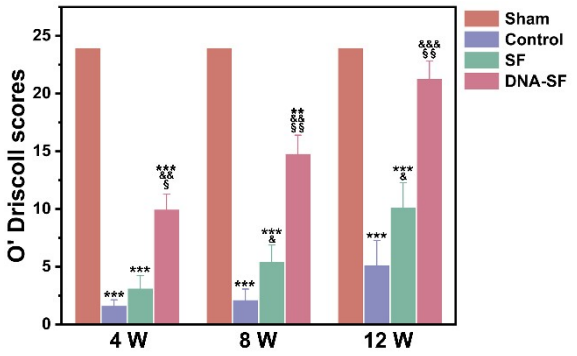

Figure S16. O'Driscoll score grading of the cartilage defect at 4, 8 and 12 weeks after treatment. (*: compared to value at Sham groups, &: compared to value at Control groups, §: compared to value at SF groups.)

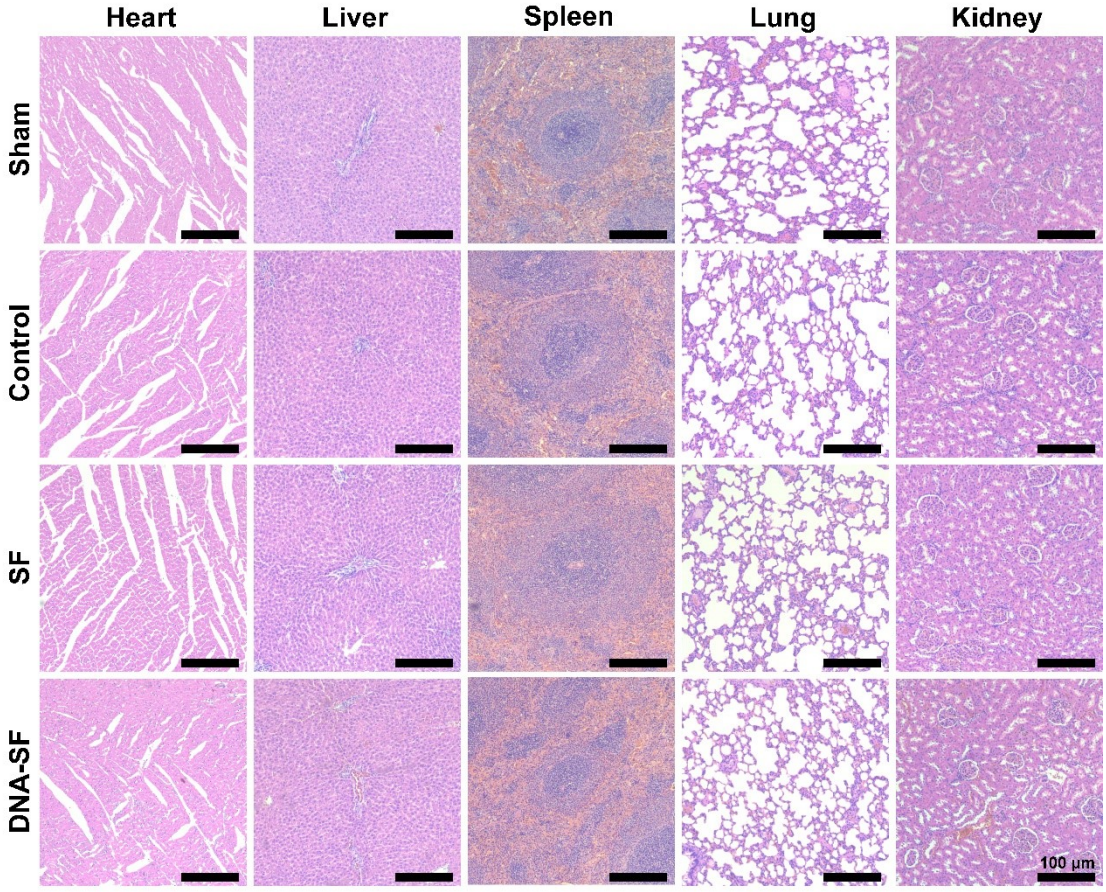

Figure S17. The toxicity of in situ nanocomposite hydrogel at 12 weeks. Scale bar: 100 μm.

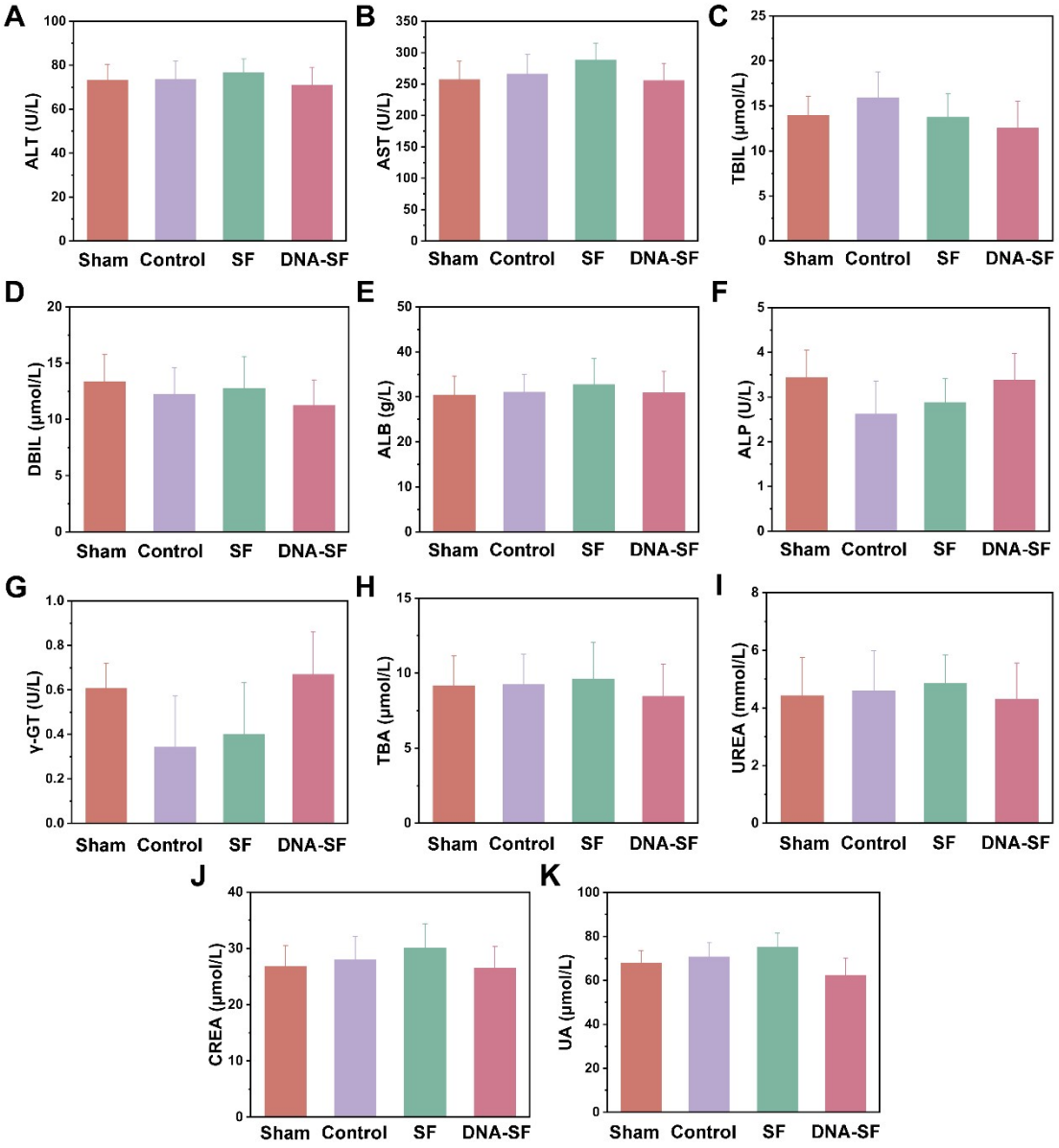

Figure S18. Liver and kidney function testing: (A) Alanine aminotransferase. (B) Aspartate aminotransferase. (C) Total bilirubin. (D) Direct bilirubin. (E) Albumin. (F) Alkaline phosphatase. (G) γ-glutamyl transferase. (H) Total bile acid. (I) Urea. (J) Creatinine. (K) Uric acid.