Supporting information for:

Chirality from D-Guanosine to L-Guanosine Shapes a Stable Gel for Three-Dimensional Cell Culture

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General Materials.

D-guanosine powders (CAS number:118-00-3) were purchased from Sigma-Aldrich (St. Louis, USA). L-guanosine powders (CAS number: 26578-09-6) were purchased from Wuhu Nuowei Chemistry Co. Ltd. Other reagents were of laboratory grade as obtained from commercial suppliers and were used without further purification. CCK-8 assay kit was purchased from DOJINDO laboratories (Japan). Live/dead viability/cytotoxicity kit (L3224) was purchased from Life Technologies Corporation (Oregon, USA). BALB/c mice (6-7 weeks) were purchased from Dashuo Technologies Corporation (Chengdu, China). The deionized water was purified by a Milli-Q purifier.

General Methods.

Mass spectrometry data was got on ESI-Q-TOF (micrOTOF-Q). Elemental analyses were performed on vario MICRO select analyser (Elementer, Germany) at Analysis and Testing Center, Sichuan University.Optical rotations determined on model 341 polarimeter (Perkin Elmer) at 20 ℃. Sodium lamp (λ=589 nm) and sample cell (L=1 dm) were used. Dimethyl sulphoxide was used as solvent. Optical rotation data were reported as follows: $[\alpha]_D^{20}$ (concentration c = g/mL, solvent). Circular dichroism spectra were recorded between 200 and 300 nm using a JASCO J-810 spectrometer. Experiments were carried out by placing the heated liquid mixture by a pipette tip onto a quartz plate with a 0.1 mm path length. That mixture would cool down to form gels on the plate. The experiments were performed with gels with a concentration of 0.5 mg/mL in a 0.2 M aqueous solution of different alkali metal ions. Nuclear Magnetic Resonance (NMR) spectra were recorded on an AV II (Bruker, Germany) spectrometer at 600 MHz. δ values in ppm are relative to Me4Si as internal standard.

Preparation of hydrogels.

Preparation of hydrogels with chloride solution: Gelator powders (D-G or L-G) mixed with 0.2 M chloride solution were heated at 80-100 ℃. Once fully dissolved, gelators then cooled to room temperature (~25 ℃) and gradually formed gels. For example, 2.8 mg gelators were weighted and mixed with 200 μL 0.2 M potassium chloride solution to obtain 1.4% w/v hydrogels.

Preparation of hydrogels with sodium borate and phosphate buffer saline (PBS): Gelator powders (D-G or L-G) mixed with 0.25 M sodium borate solution and PBS were heated at 80-100 ℃. Once fully dissolved, gelators then cooled to room temperature (~25 ℃) and gradually formed gels. For instance, 5.6 mg gelators were weighted and mixed with 160 μL PBS and 40 μL 0.25 M sodium borate solution to obtain 2.8% w/v hydrogels.

Scanning electron microscopy (SEM) imaging.

SEM was performed using a high-resolution INSPECT F50. The hydrogels were prepared in a sample tube and were frozen and evaporated using a vacuum pump thereafter. Prior to the examination, the xerogels were attached to the silica

wafer and coated with a thin layer of gold.

Rheological experiments.

Rheological experiments were performed at room temperature (~20 °C) using conical parallel plate geometry (CP50). The plate was pre-heated to 80 °C and 1 mL heated liquid mixtures were transferred onto the plate by a pipette tip. The rotor descended and pressed the liquid into a disk with a 0.5 mm gap space. Oil was added along the plate to minimize the effect of evaporation. Temperature of the plate was then controlled to fall down to the room temperature. Parameters were set according to tests.

CCK-8 assay.

Normal oral keratinocyte (NOK) cells were provided as part of cell collection of the State Key Laboratory of Oral Diseases, West China College of Stomatology, Sichuan University.

CCK-8 assay on gelators: A volume of 200 μL NOK cells were planted into 96-well plate with a cell density of 2~4*10^4/mL and incubated over night at 37 °C in a CO_2 incubator. Gelator were dissoved with pure K-SFM and added into the plate. After incubated for 24 hours, optical density values were determined via microplate reader at the wave length of 450 nm.

CCK-8 assay on hydrogels: A volume of 1mL NOK cells were planted into 24-well plate with a cell density of 2~4*10^5/mL and incubated over night at 37 °C in a CO_2 incubator. Heated liquid mixtures were transferred into a trans-well chamber by a pipette tip and cooled down at room temperature for ten minutes to form gels (2.8% w/v). After the trans-well containing gels were transferred into the plate and incubated for 3 days or 6 days, optical density values were determined via microplate reader at the wave length of 450 nm.

Culture medium was changed every 3 days. Data were analysed by program IBM SPSS.

In vivo Degradation Study and Histopathological Analysis.

All animal experimental protocols were approved by the animal care and use regulations (Ethics Committee of West China Hospital of Stomatology, Sichuan University), and the experiments were carried out under control of the University's Guidelines for Animal Experimentation. BALB/c mice (6-7 weeks) were acclimatized for 7 days after arrival. Then mice were subcutaneously injected with a gel (1 mL 2.8% w/v D-G or L-G gels). Mice treated with 800 μL PBS and 200 μL 0.25 M $NaBO_3$ solution were used as control group. Photos were taken at different time points (0h, 3h, 6h, 12h, 24h, 48h, 72h). Meanwhile, tissues (including heart, liver, spleen, lung and kidney) were taken to Haemotoxylin and Eosin staining.

Three-dimensional cell culture.

A volume of 50 μL NOK cell suspension (1~2*10^5/mL) was mixed with 100

μL heated liquid mixture and the transferred into a culture slide. Gels formed 10 minutes later and another 50 μL culture medium was added along and on the gels. Culture medium was changed every day. After incubated at 37 ℃ in a humidified incubator containing 5% CO_2 for 6 days, cells were stained with Live/dead staining assay following instructions and photographed under a confocal microscope. Live/dead cells were counted by using program Bitplane Imaris. Percentage of living cells was calculated and analysed by program IBM SPSS.

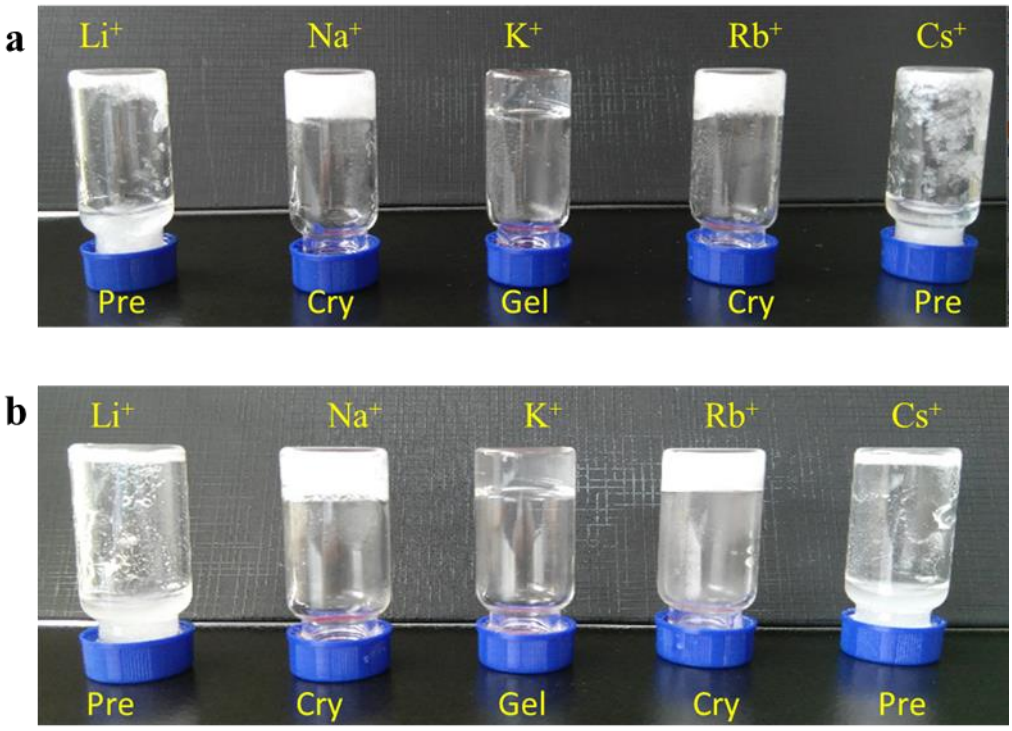

Figure S1. Inversion tests of 1.4% w/v D-G (a) and L-G (b) gels with different metal ions.

Table S1. Gelation properties of hydrogels obtained from D-G and L-G under different conditions as mentioned below

Metal ions	Gelation	Minimum gelation concentration (mgc) (%, w/v)	Crystallization*	Lifetime stability (at room temperature)	Thermo-reversibility*	T-gel (°C)
D-G						
Li^+	Pre	—	—	—	—	—
Na^+	Gel	0.93	Y	<1h	Y	45-50
K^+	Gel	0.47	Y	<1d	Y	60-65
Rb^+	Gel	0.70	Y	2h	Y	40-45
Cs^+	Pre	—	—	—	—	—
L-G						
Li^+	Pre	—	—	—	—	—
Na^+	Gel	0.93	Y	<1h	Y	35-40
K^+	Gel	0.56	Y	17-20d	Y	65-70
Rb^+	Gel	0.70	Y	<1h	Y	35-40
Cs^+	Pre	—	—	—	—	—

*Y indicates yes (gel crystallizes or gel has thermo-reversibility); h: hour(s); d: day(s). Except the mgc values, the other values were measured at the concentration of 1.4% w/v of gelators and 0.2M of metal ions.

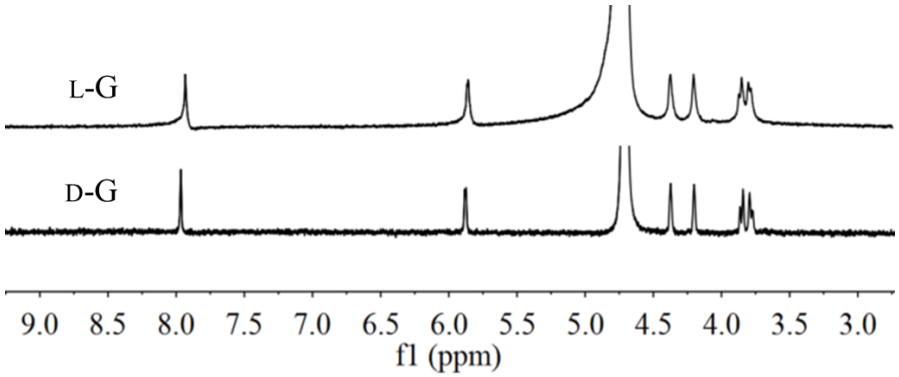

Figure S2. ^{1}H NMR spectrum of D-G and L-G in D_2O at room temperature. No impurity peak was observed.

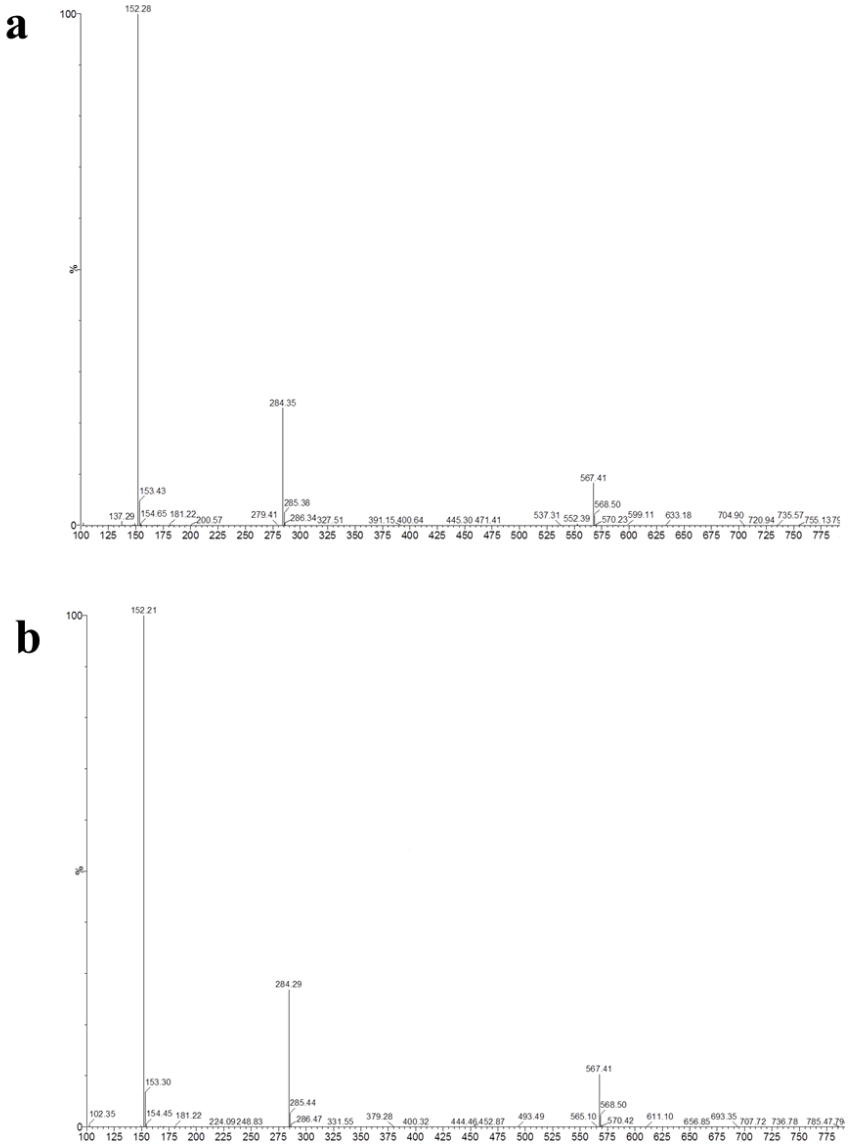

Figure S3. Mass spectra of D-G (a) and L-G (b) in H_2O at room temperature. No impurity peak was observed.

Table S2. Elemental analysis of D-G and L-G.

	Required			Founded		
	C (%)	**N (%)**	**H (%)**	**C (%)**	**N (%)**	**H (%)**
D-G ($C_{10}H_{13}N_5O_5 \cdot H_2O$)	39.87	23.25	5.02	39.26	22.98	4.98
L−G ($C_{10}H_{13}N_5O_5 \cdot H_2O$)	39.87	23.25	5.02	39.49	23.11	5.11

Table S3. Optical rotation test of D-G and L-G.

	Optical rotation
D-G	$\{[\alpha]_D^{24}$=-38.92±0.38 (c=0.025, DMSO)$\}$
L-G	$\{[\alpha]_D^{24}$=+38.83±0.52 (c=0.025, DMSO)$\}$

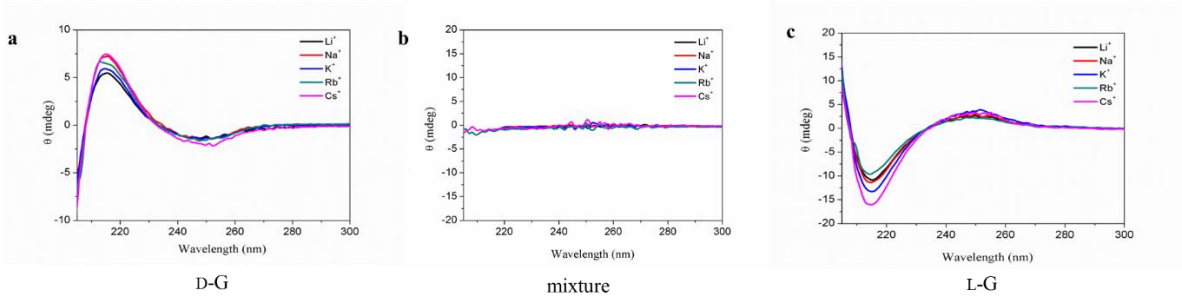

Figure S4. CD spectra. (a) D-G exhibited positive peaks at the wavelength of 215 nm. (b) Mixture at the rate of 1:1 showed no peaks. (c) L-G exhibited negative peaks at the wavelength of 215 nm. D-G and L-G showed opposite chirality.

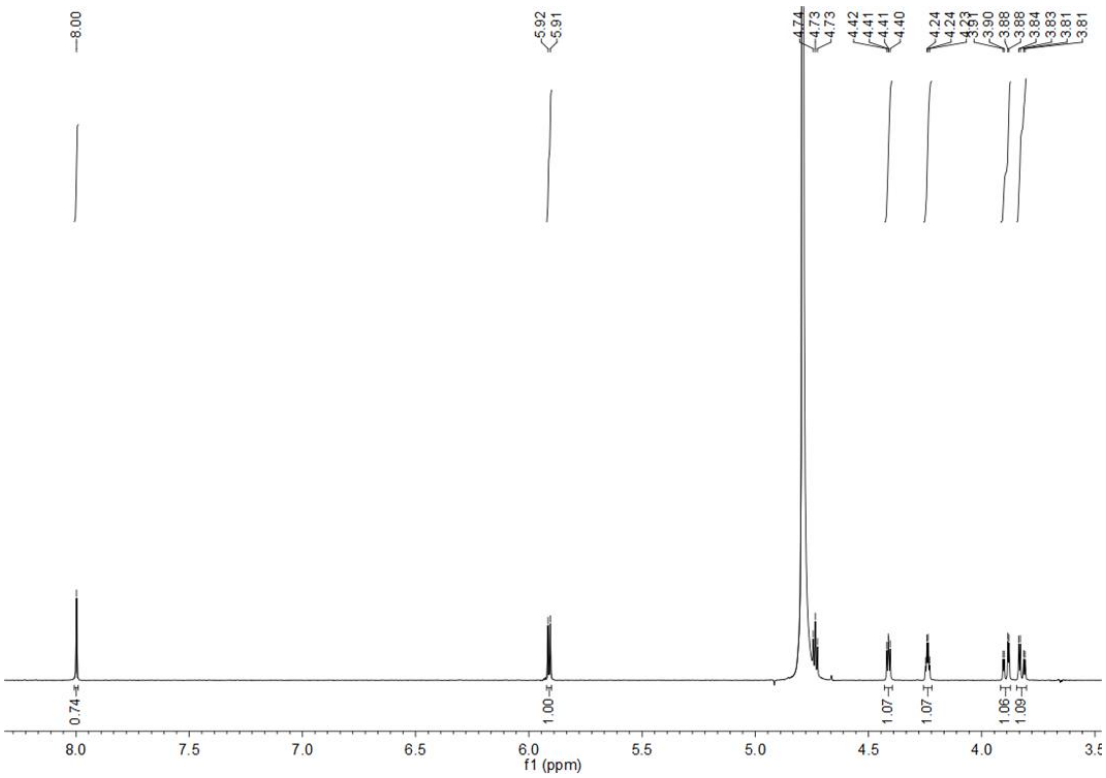

Figure S5. ^{1}H NMR spectrum of D-G+KCl in D_2O at room temperature.

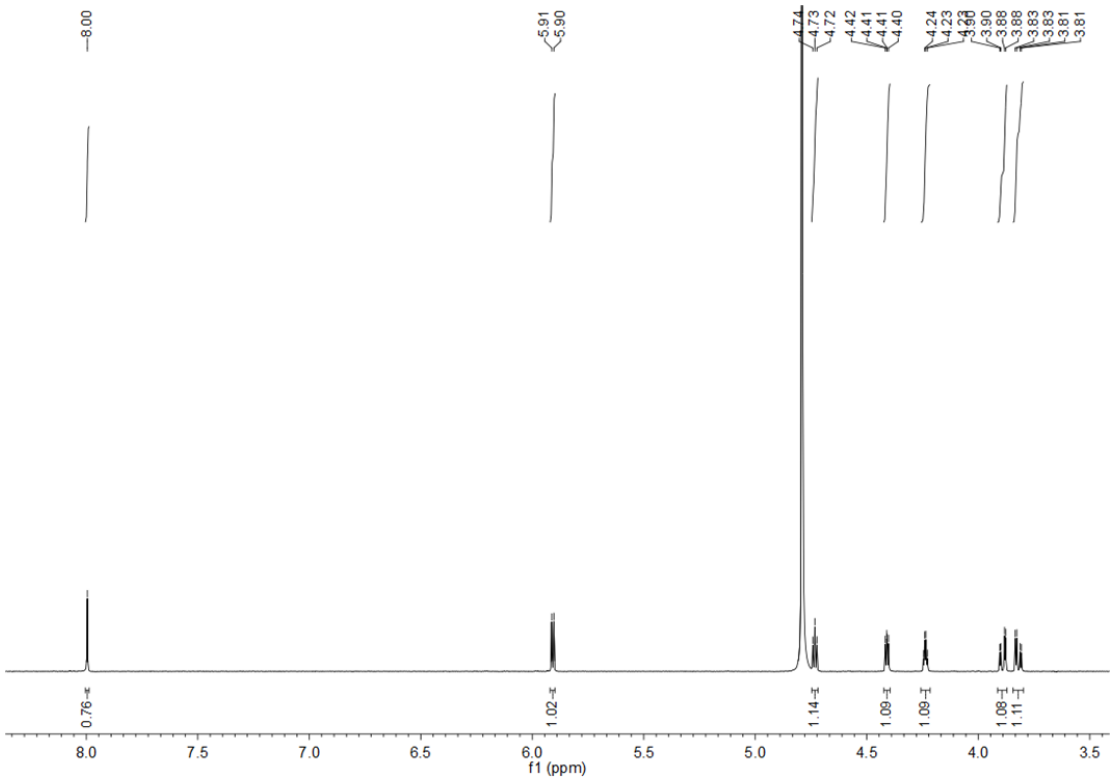

Figure S6. ^{1}H NMR spectrum of L-G+KCl in D_2O at room temperature.

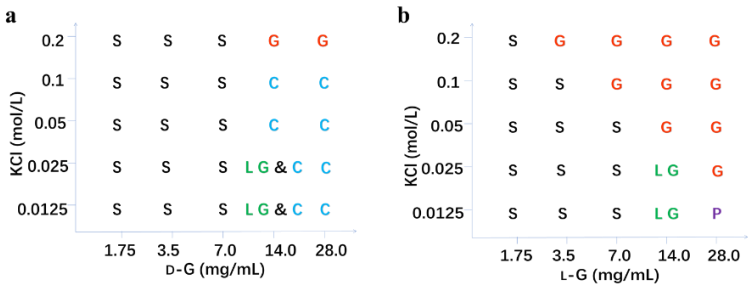

Figure S7. Phase diagram of the hydrogel formation ability at different concentrations of K^+ and L-G and D-G gels: G: gel, S: solution, C: crystal, L G: loose gel, P: precipitate.

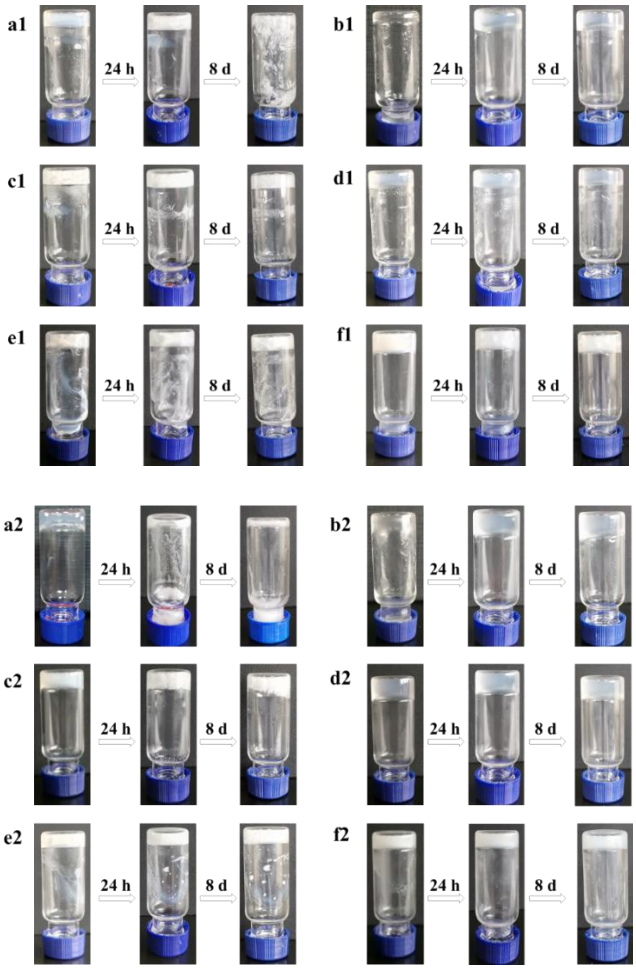

Figure S8. Inversion tests of D-G (a1, b1, c1, d1, e1, f1) and L-G (a2, b2, c2, d2, e2, f2) gels with PBS and sodium borate at 0.7% w/v (a1, b1, a2, b2), 1.4% w/v (c1,d1, c2, d2), 2.8% w/v (e1, f1, e2, f2). D-G and L-G without borate crystalized and collapsed within ten minutes or one hour. Although 1.4% w/v D-G and L-G gels collapsed partly in the fifth minute, they formed firmer gels later. Meanwhile, 5.6% w/v D-G and L-G gels crystalized within 24 hours, which may be attributed to that relatively excessive gelator molecules could find enough borates to form borate diesters. Crystals were observed in 2.8% w/v D-G gels within 24 hours and in 2.8% w/v D-G gels on day 17.

Table S4. Gelation properties of hydrogels obtained from D-G/L-G+$NaBO_3$+PBS gels under different conditions as mentioned below

Gelators	Gelation	Minimum gelation Concentration (%, w/v)	Crystallization*	Lifetime stability (at room temperature)	Thermo-reversibility*	T-gel (℃)
D-G	Gel	0.93 (PBS+$NaBO_3$) 0.7 (PBS)	Y	< 24 hours	Y	70-75
L-G	Gel	1.12 (PBS+$NaBO_3$) 0.8(PBS)	Y	17 d	Y	60-65

*Y indicates yes (gel crystallizes or gel has thermo-reversibility); h: hour(s); d: day(s). Except the mgc values, the other values were measured at the concentration of 2.8% w/v of gelators.

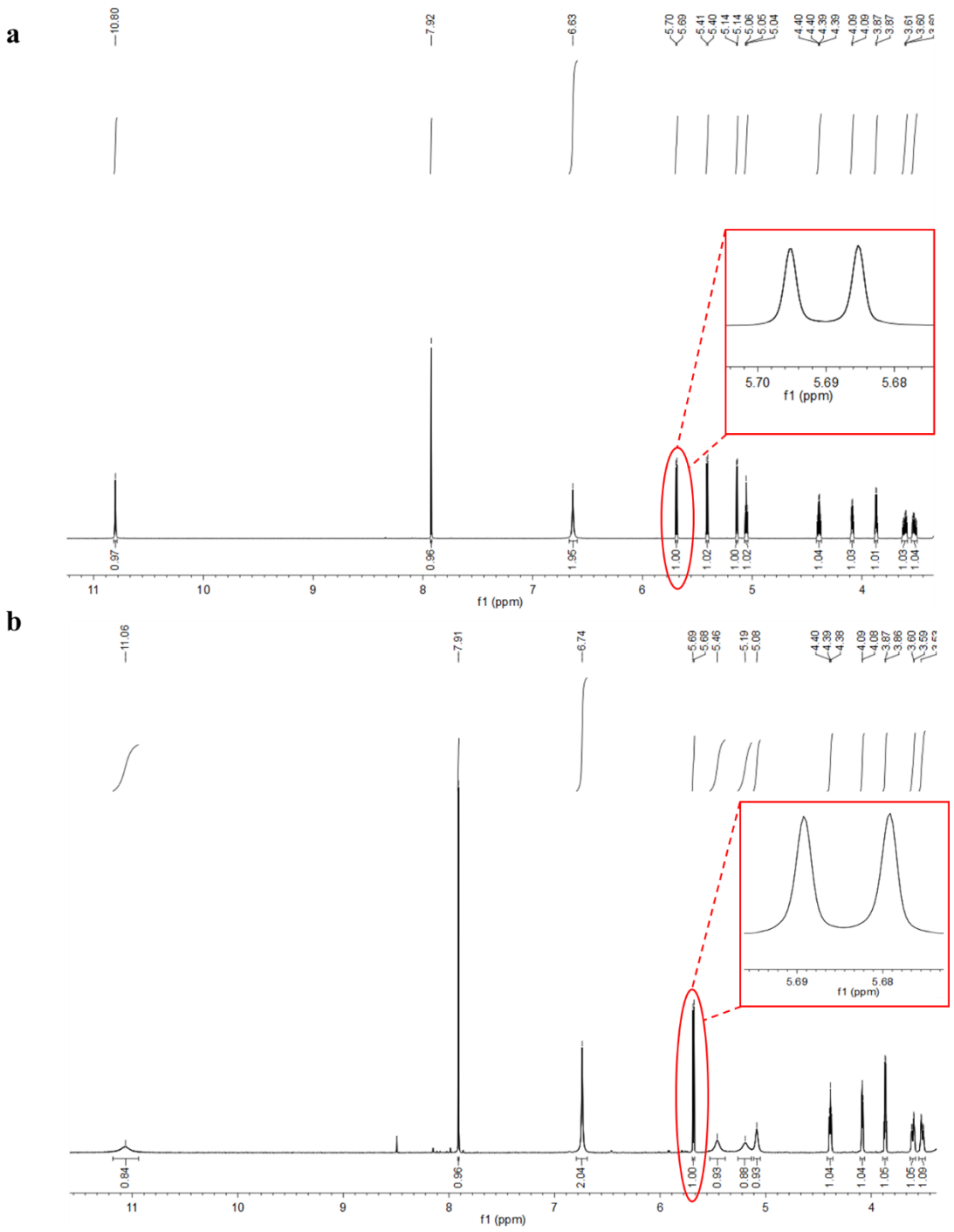

Figure S9. ^{1}H NMR spectra of D-G+KCl (a) and L-G+KCl (b) gels in DMSO at 25 ℃.

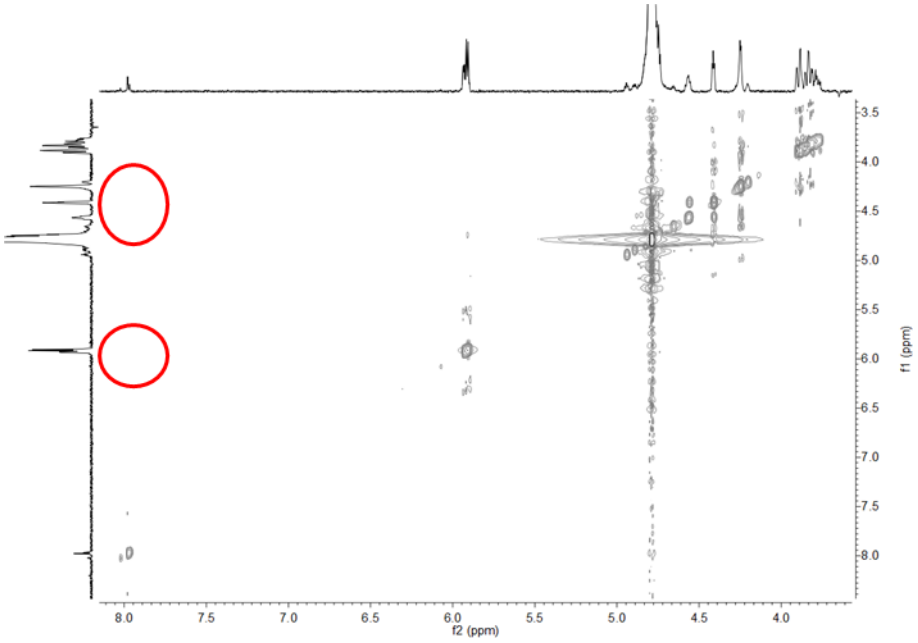

Figure S10. NOE spectrum of D-G+$NaBO_3$+PBS in D_2O at room temperature.

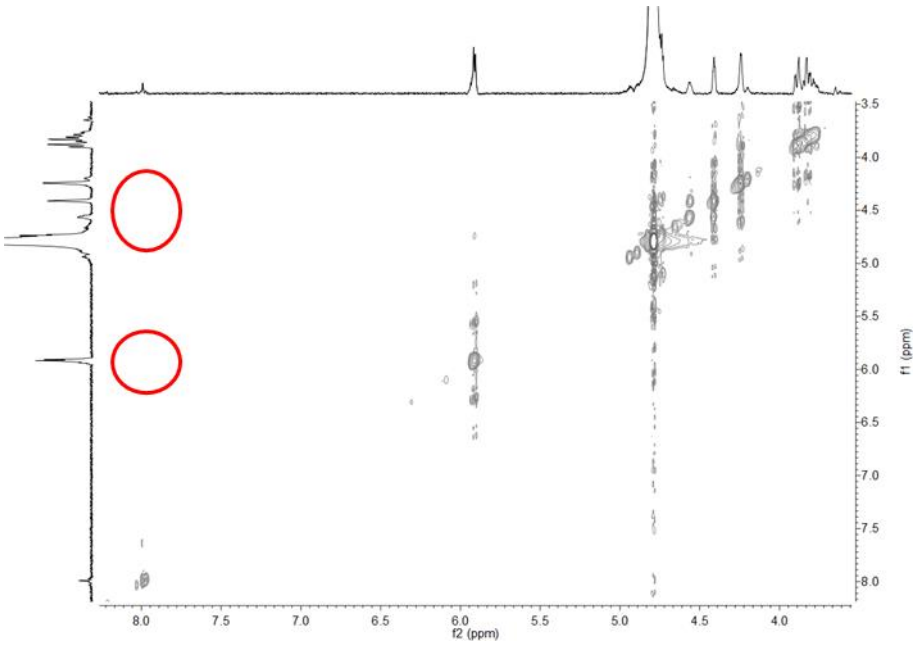

Figure S11. NOE spectrum of D-G+KBO_3+PBS (b) in D_2O at room temperature.

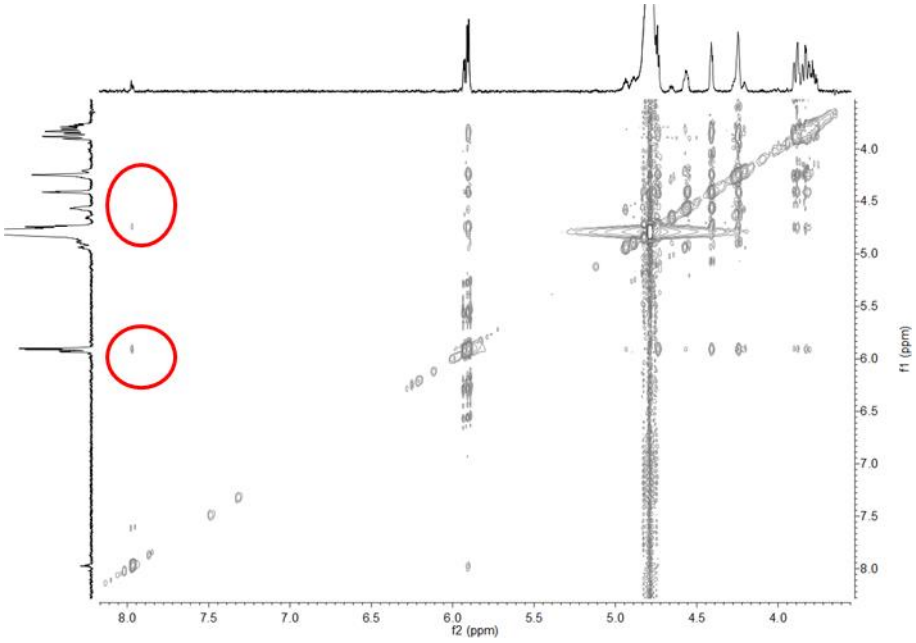

Figure S12. NOE spectrum of L-G+$NaBO_3$+PBS in D_2O at room temperature.

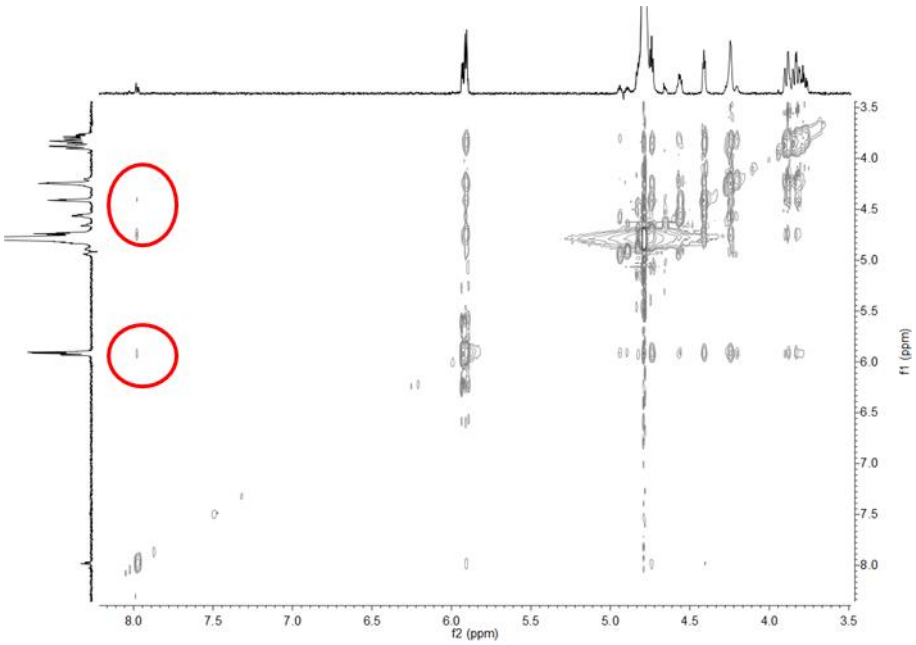

Figure S13. NOE spectrum of L-G+KBO_3+PBS in D_2O at room temperature.

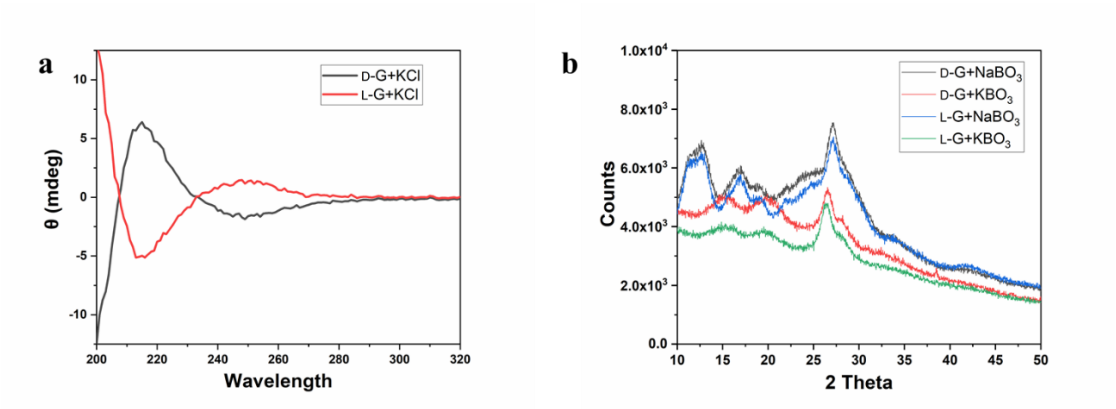

Figure S14. (a) CD spectrum of D-G (0.028% w/v) and L-G (0.014%) in KCl solution. (b) PXRD spectrum of D-G and L-G xerogels at concentration of 2.8% w/v in D_2O.

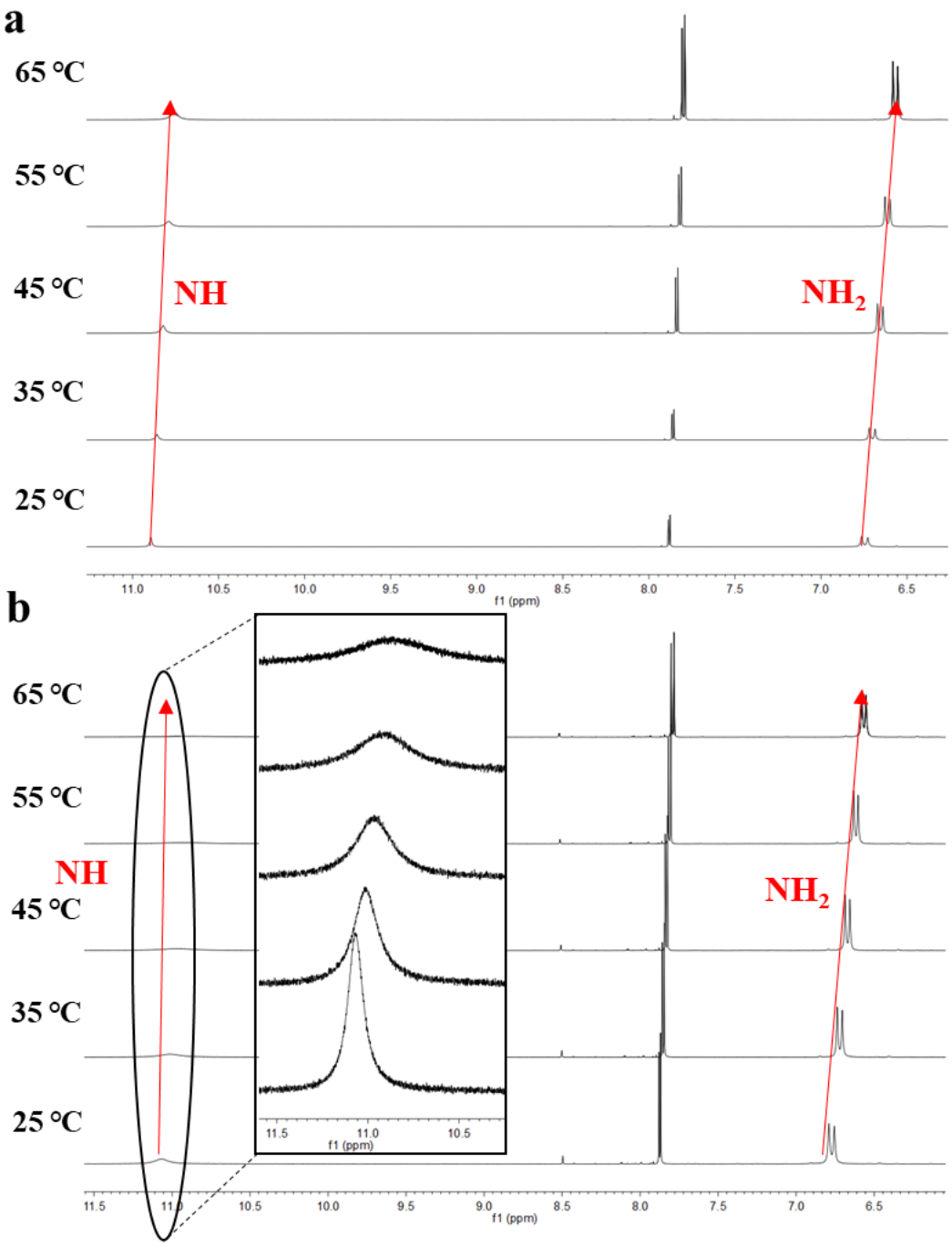

Figure S15. (a) ^{1}H VT-NMR spectrum of D-G with borates in DMSO recorded from 25 to 65 °C. The chemical shift for NH of D-G from δ 10.89 ppm to δ 10.71 ppm (65 °C) with Δδ = 0.13 ppm and for NH_2 of D-G from δ 6.76 ppm to δ 6.58 ppm (65 °C) with Δδ = 0.18 ppm, suggesting that the hydrogen atoms (NH and NH_2) participating in the formation of intermolecular hydrogen bonds in D-G gels. (b) ^{1}H VT-NMR spectrum of L-G with borates in DMSO recorded from 25 to 65 °C. The chemical shift for NH of L-G from δ 11.07 ppm to δ 10.87 ppm (65 °C) with Δδ = 0.20 ppm and for NH_2 of L-G from δ 6.79 ppm to δ 6.58 ppm (65 °C) with Δδ = 0.21 ppm, suggesting that the hydrogen atoms (NH and NH_2) participating in the formation of intermolecular hydrogen bonds in L-G gels.

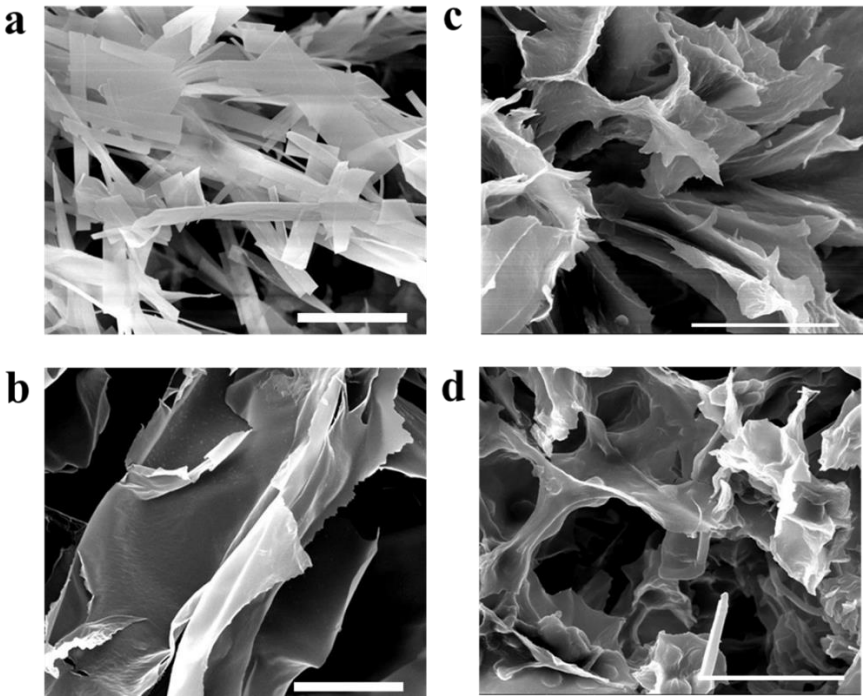

Figure S16. SEM images of xerogels. (a)(b) D-G+KCl (a) and L-G+KCl (b) gels at the concentration of 2.8% w/v (scale bar: 15 μm). (c)(d) D-G+NaBO3+PBS (c) and L-G+NaBO3+PBS (d) gels at the concentration of 2.8% w/v (scale bar: 20 μm).

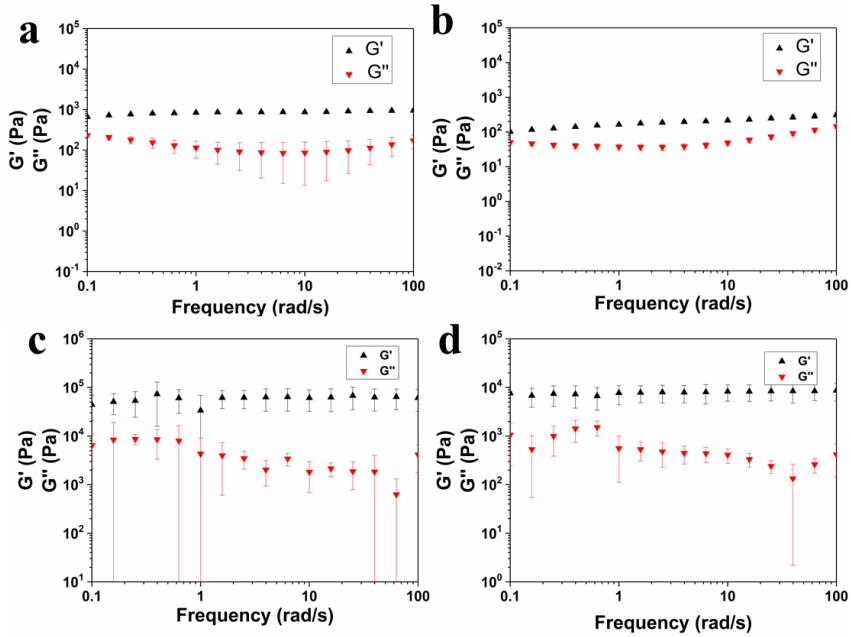

Figure S17. Frequency sweep test. G' at the angular frequency of 0.1 rad/s of D-G gel with KCl was 662.29 Pa (95% CI: 617.49 Pa, 707.08 Pa) (a), of L-G gel with KCl was 101.49 Pa (95% CI: 96.791 Pa, 106.18 Pa) (b), of D-G gel with $NaBO_3$ was 44287 Pa (95% CI: 11337 Pa, 77236 Pa) (c), of L-G gel with $NaBO_3$ was 7580.4 Pa (95% CI: 6370.7 Pa, 8430 Pa) (d); Data were analyzed by a Bonferroni multiple comparisons test after a significant ANOVA ($P < 0.05$).

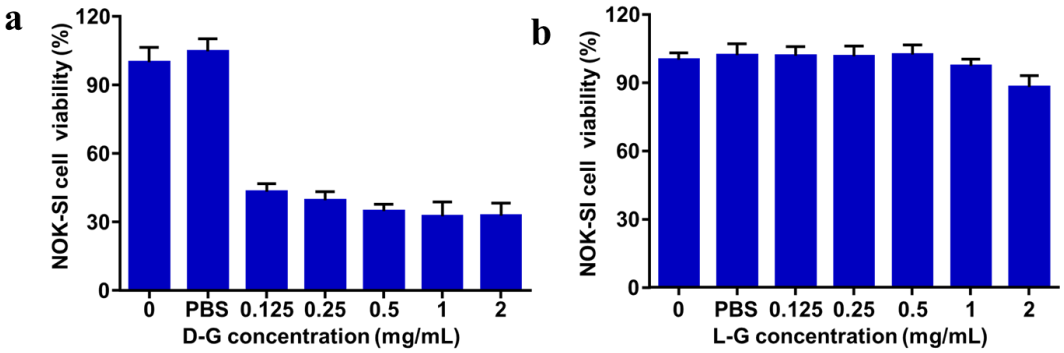

Figure S18. CCK-8 assay of D-G and L-G at different concentrations.

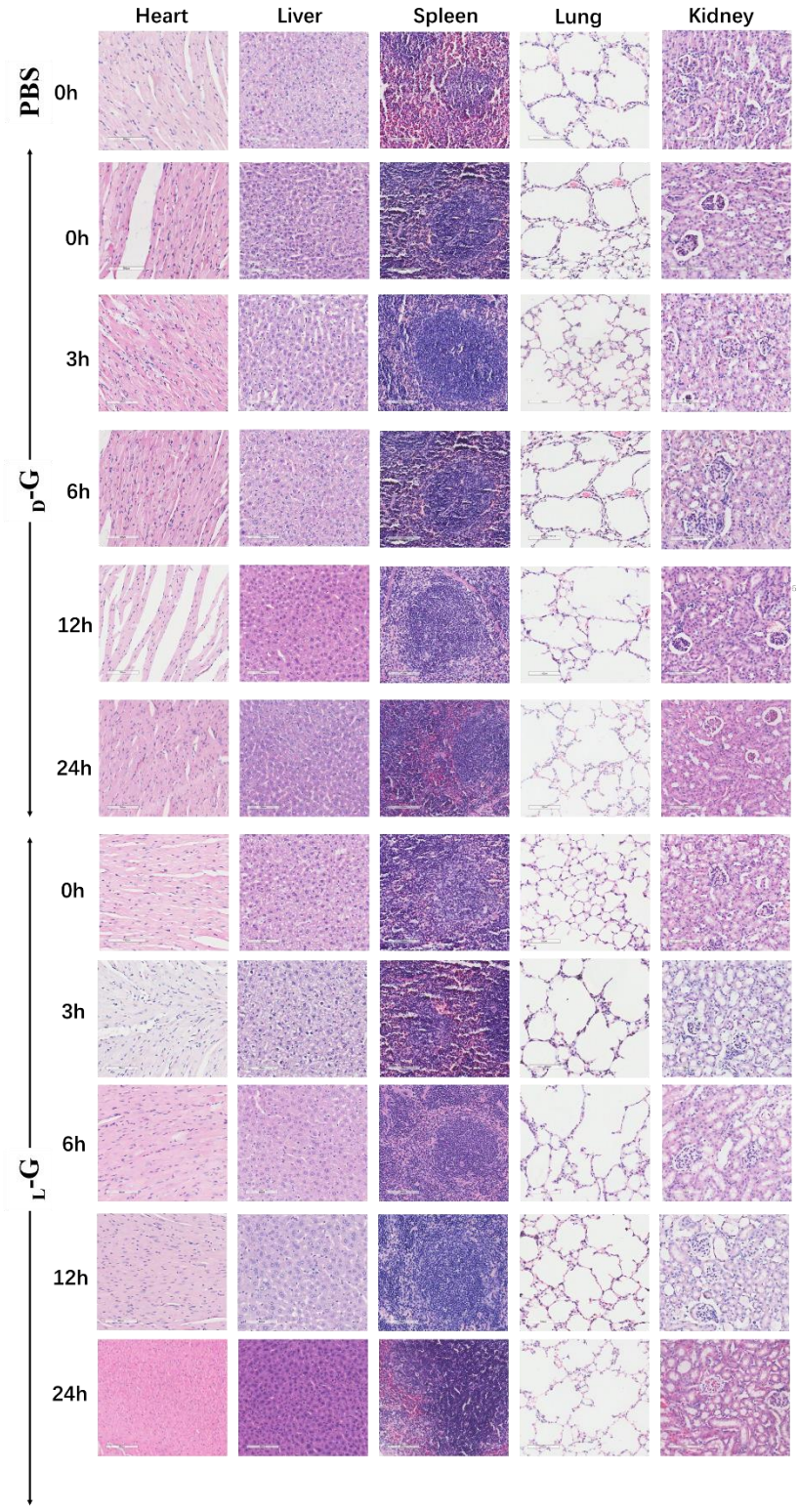

Figure S19. HE images of control group and gels' group. The scale bar is 100 μm.