Electronic Supplementary Information

Charge Reversal Induced Colloidal Hydrogel Acts as a Multi-stimuli Responsive Drug Delivery Platform for Synergistic Cancer Therapy

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Experimental Procedures

Reagents and Chemicals. Gelatin type A (from porcine skin, powder, 300 Bloom) and dopamine hydrochloride (> 98%) were obtained from Sigma-Aldrich. Glucono delta-lactone (GDL, > 99.0%) was bought from Aladdin Industrial Corporation. Doxorubicin hydrochloride (DOX) was purchased from Beijing Huafeng United Technology Co., Ltd. All chemicals were used as received without additional purification.

Synthesis of Gela and PDA NPs. Gela NPs were prepared through a typical two-step desolvation method reported elsewhere. Uniform PDA NPs with average diameter around 100 nm were developed by using a self-polymerization method. Briefly, concentrated ammonia aqueous solution (28~30%, 5.0 mL) was added into the mixture of deionized water (DI water, 90 mL) and ethanol (20 mL) under room temperature. Then, dopamine hydrochloride aqueous solution (50 mg/mL, 10 mL) was quickly added into the above suspension and uniform PDA NPs could be obtained after 24 h stirring.

Synthesis of Colloidal Hydrogel. Certain amount of Gela and PDA NPs were homogeneously mixed at a basic condition (pH ≈ 12) and subsequently added with GDL powder (50 mg) under magnetic stirring to initiate the gelation. For DOX-loaded

hydrogel, certain amount of DOX was firstly mixed with PDA NPs and then followed the procedure of colloidal hydrogel fabrication.

Characterization. The morphogies and hydrodynamic diameter of as-prepared Gela NPs, PDA NPs and colloidal hydrogel were acquired by scanning electron microscopy (SEM, Hitachi SU8020, Japan) and dynamic light scattering instrument (Zetasizer Nano-Series, Malvern Instruments Ltd.), respectively. The viscoelasitic properties of colloidal hydrogels were characterized by an TA Discovery DHR-3 rheometer with a flat steel plate geometry (40 mm diameter). The NIR laser induced temperature increase of collodial hydrogel was monitored by an IR thermal camera (Fluke, USA).

Cytotoxicity of Colloidal Hydrogel. The two components (Gela and PDA NPs) and leaching solution of colloidal hydrogel were used to incubate with normal HUVECs to evaluate the cytotoxicity *in vitro* by a typical MTT assay. The colloidal Gela/PDA hydrogel was further subcutaneously injected into the mice for two weeks and then standard Masson's trichrome and H&E staining to detect any formation of colloagen capsule and inflammatory/pathological change around the implatation site. After the sacrifice of hydrogel-implated mice, the hematological parameters were analyzed in comparison to healthy mice with same age.

***In Vitro* Multi-Stimuli Responsive DOX Release Properties.** The DOX release profiles from colloidal Gela/PDA/DOX hydrogel under various stimuli (acidic pH, high concentration of enzyme and NIR light irradiation) were acquired by a standard dialysis method. Specially, enzymatic degradation of colloidal hydrogel was also monitored by incubating hydrogel with solution of proteinase and the released protein was quantitatively calculated by a typical bicinchoninic acid (BCA) assay.

Synergistic Cancer Cell-Killing Effect. The heat promoted cellular uptake of DOX was firstly quantitatively investigated by a fluorescence activated cell sorting (FACS) analysis. After that, *in vivo* synergistic tumor inhibition effect was performed by treating BALB/c mice bearing 4T1 tumor (~30 mm^3) under the approval of the Institutional Animal Care of Anhui Medical University (LLSC20150134) and Use Committee. After various treatment, the relative tumor volume and body weight of treated mice were recorded every other day.

Statistical Analysis. The data obtained here are analyzed by student t-tests with a setting significance of $p < 0.05$ (*) and $p < 0.01$ (**).

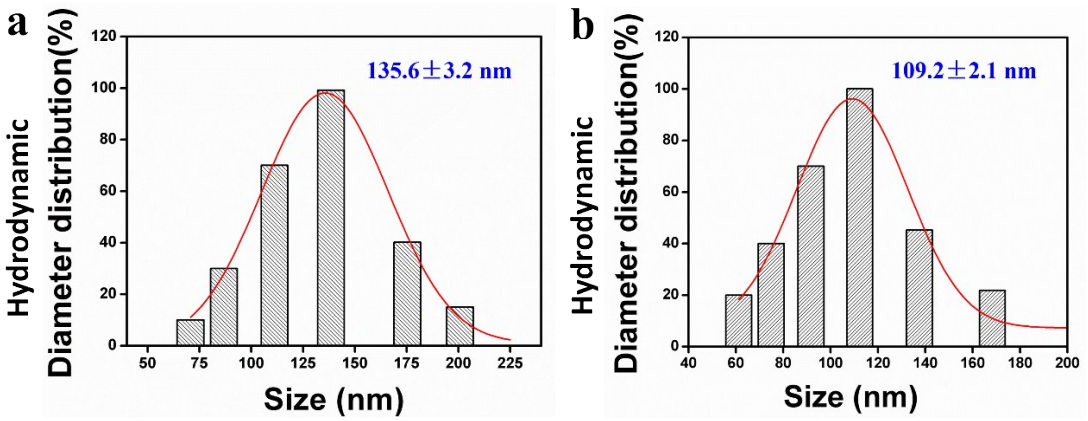

Fig. S1 Hydrodynamic diameter distribution of as-prepared a) Gela NPs (PDI: 0.12) and b) PDA NPs (PDI: 0.08).

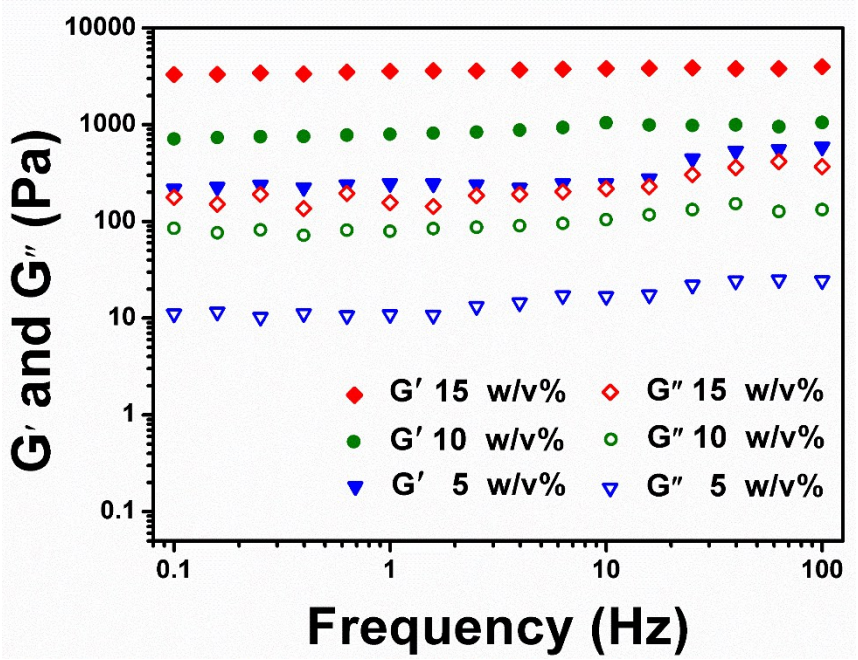

Fig. S2 Frequency dependence of elastic (G‘) and viscous (G‘‘) moduli of colloidal Gela/PDA hydrogels with different solid contents.

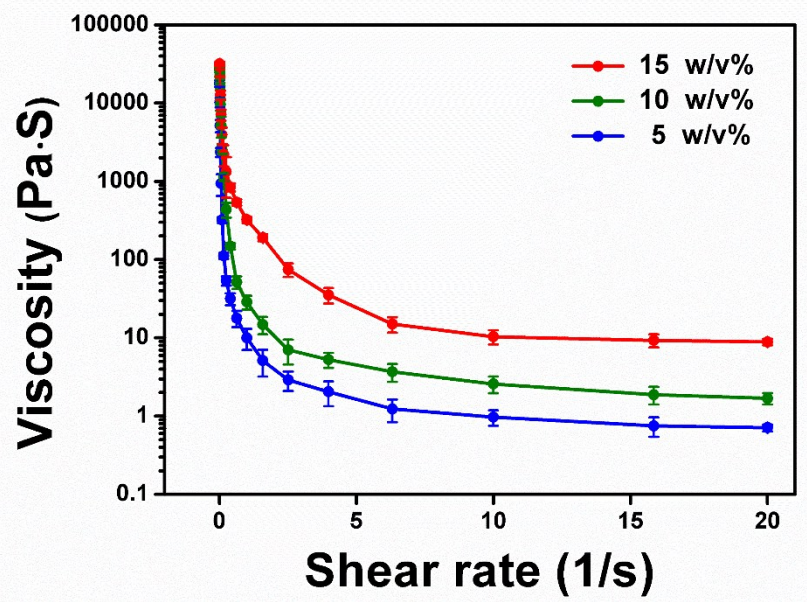

Fig. S3 Shear-thinning behaviors of colloidal Gela/PDA hydrogels with different solid contents.

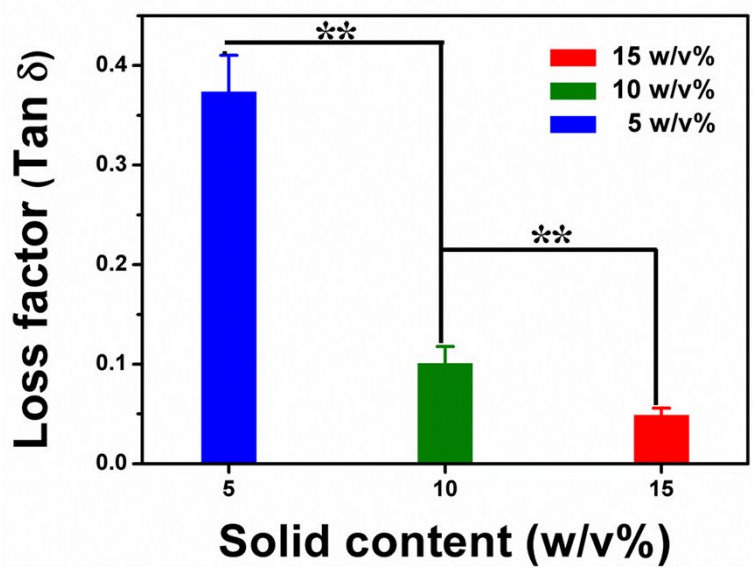

Fig. S4 Loss factor (tanδ) of colloidal Gela/PDA hydrogels with different solid contents (** p<0.01).

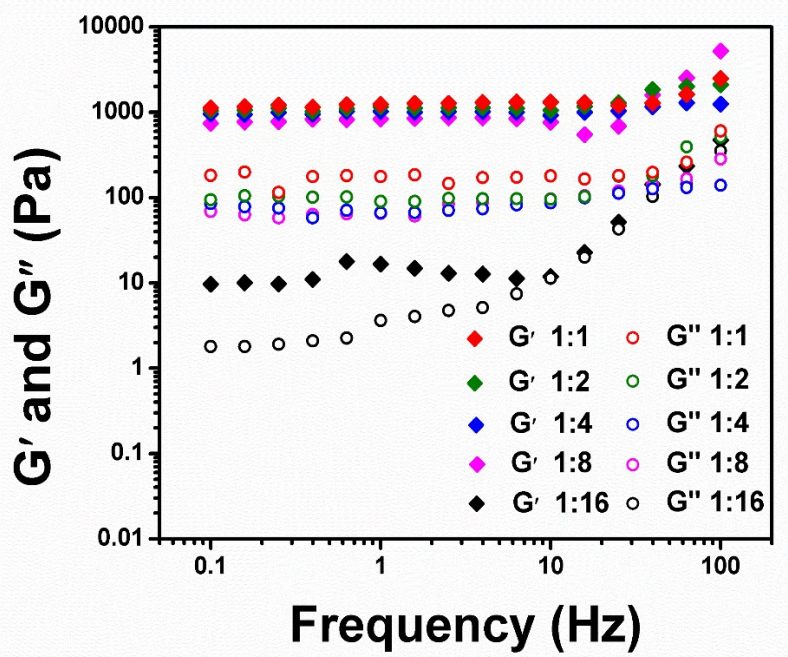

Fig. S5 Frequency dependence of colloidal Gela/PDA hydrogels with different weight ratio of PDA to Gela NPs.

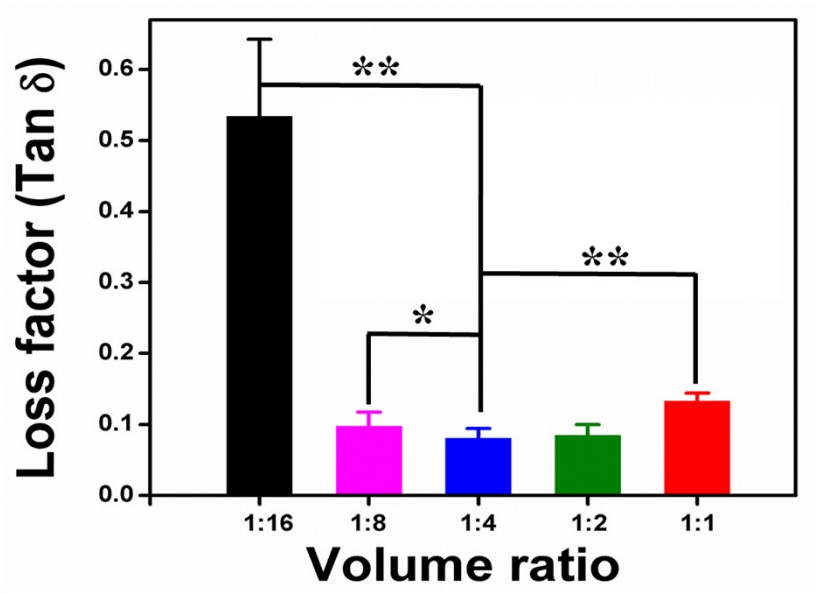

Fig. S6 Loss factor (tanδ) of colloidal Gela/PDA hydrogels with different volume ratio of PDA to Gela NPs (* p<0.05, ** p<0.01).

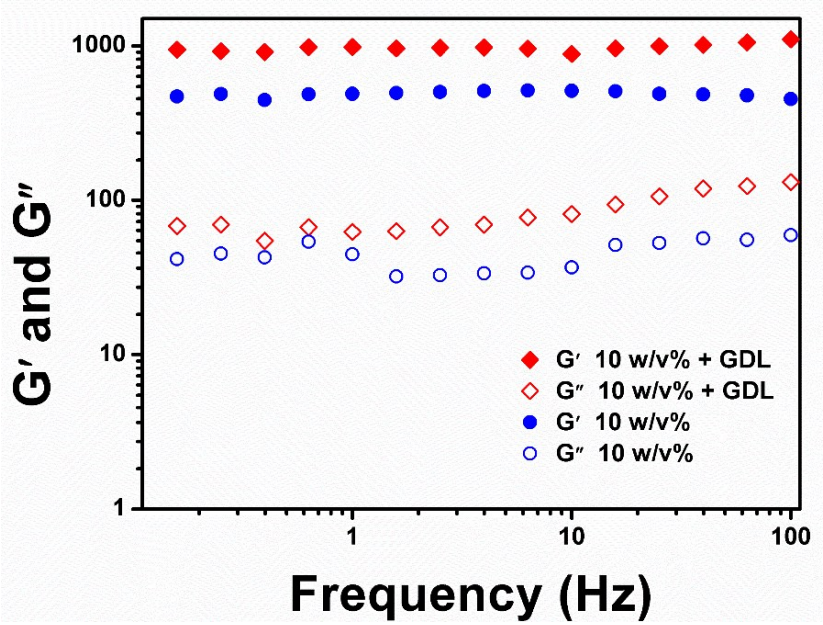

Fig. S7 Elastic G‘ and viscous G‘‘ moduli of hydrogel (10 w/v% solid content) with/without GDL.

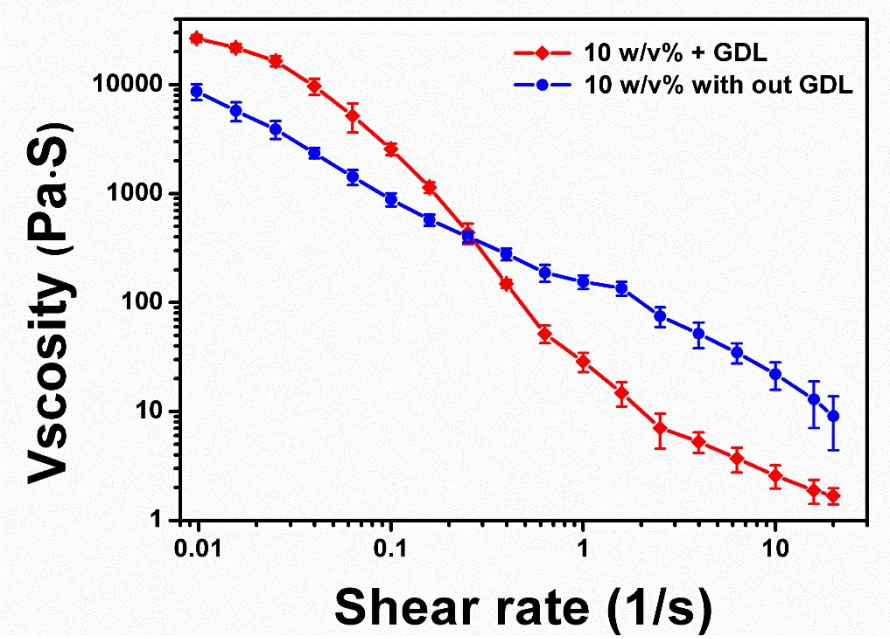

Fig. S8 Shear-thinning behavior of hydrogel (10 w/v% solid content) with/without GDL.

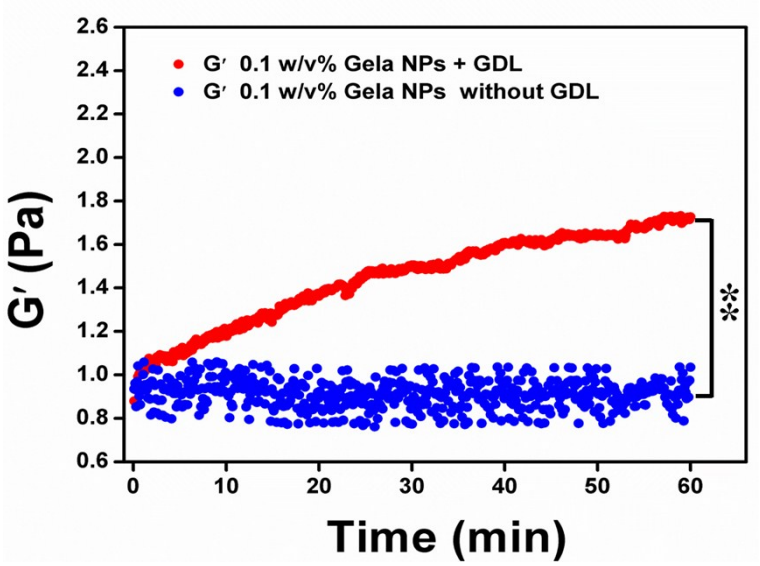

Fig. S9 Influence of GDL on the elasticity of Gela NPs (** p<0.01).

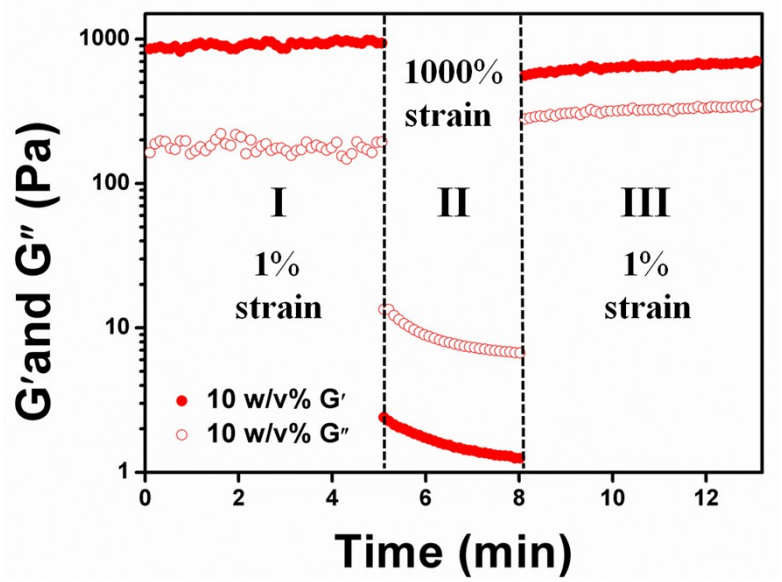

Fig. S10 Gel recovery after destruction assessed by monitoring G' and G'' as a function of time: region I, II and III shows initial gel strength, gel destruction and gel recovery, respectively.

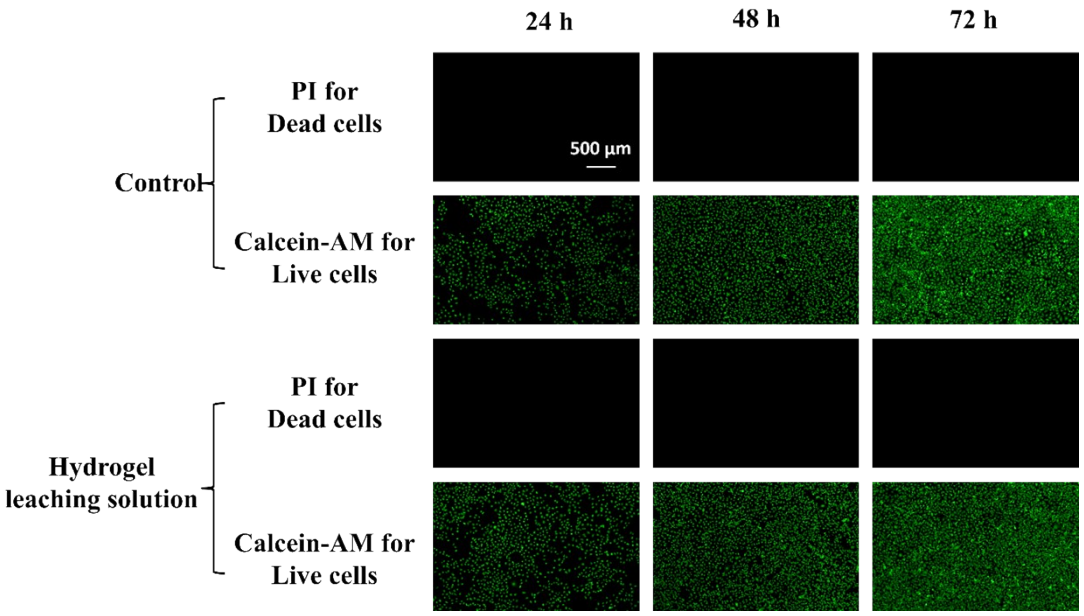

Fig. S11 Typical Live/Dead fluorescent assay for 4T1 cancer cells incubated with colloidal Gela/PDA hydrogel leaching solution.

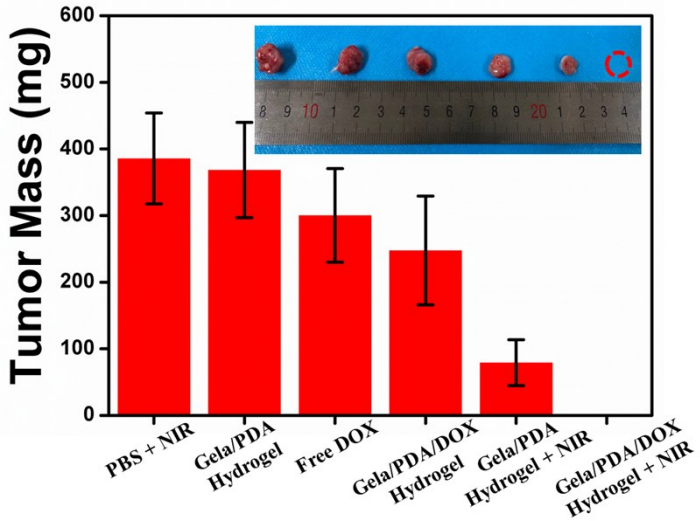

Fig. S12 Weight and typical photograph (inset) of tumors extracted from different groups.

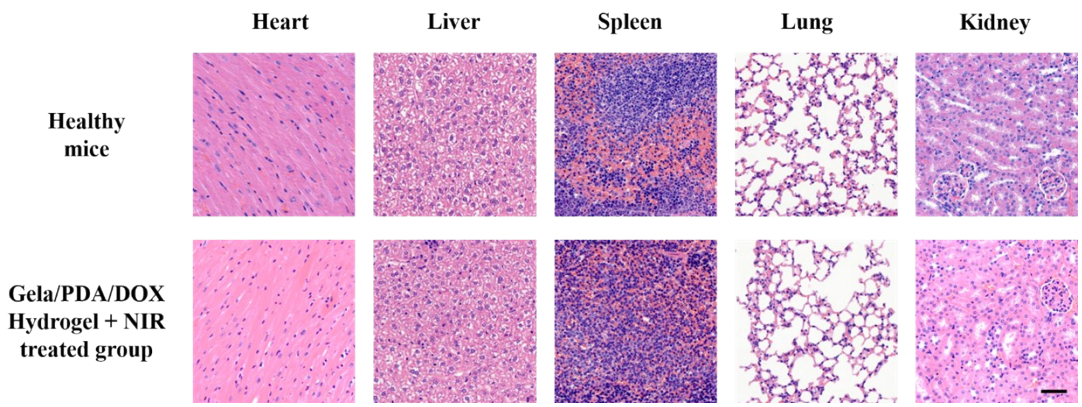

Fig. S13 Representative H&E images of major organs (heart, liver, spleen, lung and kidney) of representative mice from healthy group and Gela/PDA/DOX hydrogel + NIR group. Scale bar is 50 μm.