In situ injection of dual-delivery PEG based MMP-2 sensitive hydrogels for enhanced tumor penetration and chemo-immune combination therapy

Jianqin Yan[a,b], Zhuangzhuang Zhang[b], Xiaohui Zhan[b], Keqi Chen[c], Yuji Pu[b], Yan Liang[a], Bin He[b]*

a Department of Pharmaceutics, School of Pharmacy, Qingdao University, Qingdao 266073, China

b National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, China

c Department of Clinical laboratory, Qingdao special servicemen recuperation center of PLA navy, Qingdao 266021, China

* To whom correspondence should be addressed, E-mail: liangyan072@foxmail.com (Y. Liang)

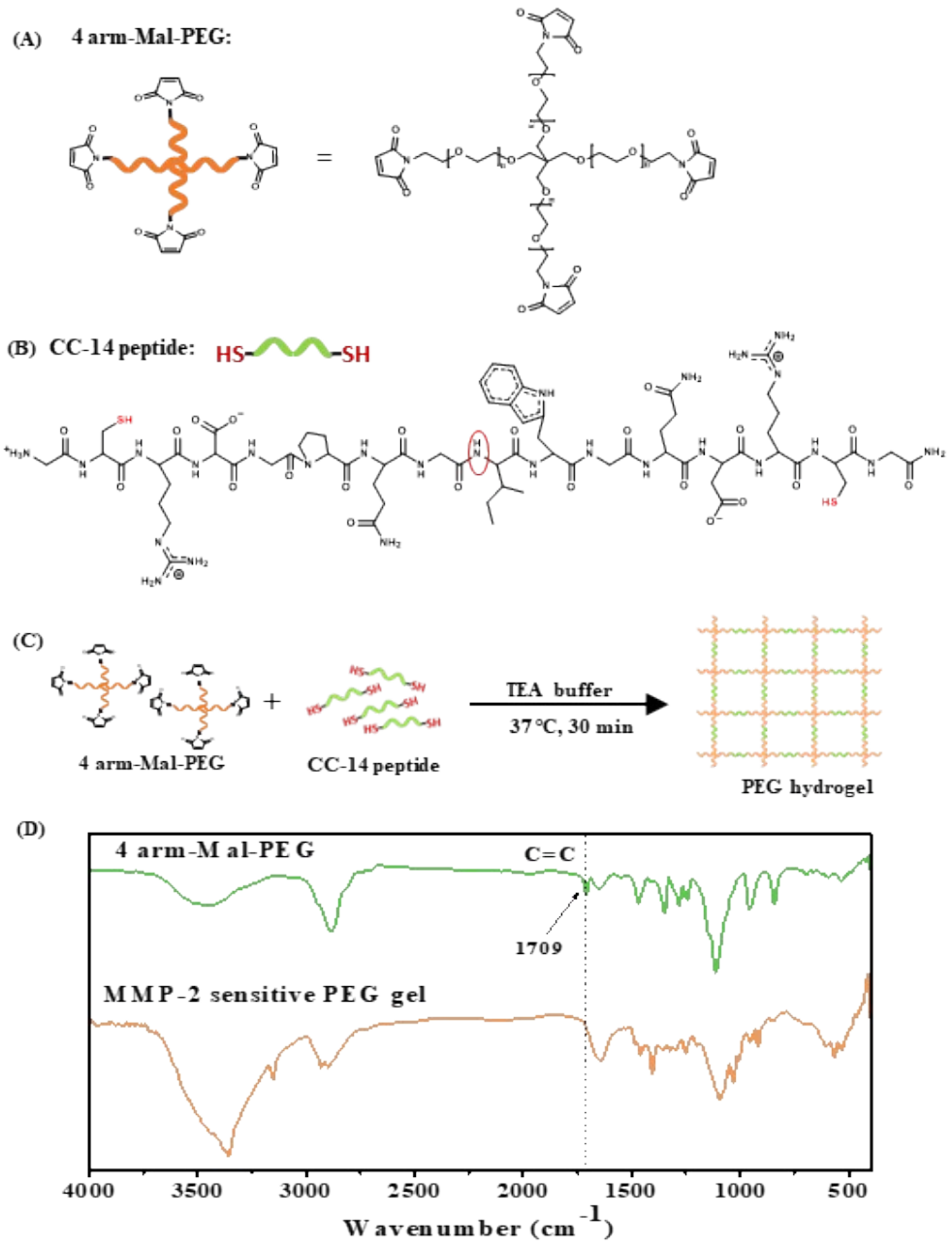

Fig. S1. Structure of 4 arm-Mal-PEG (A) and MMP-2 sensitive CC-12 peptide (B), (C) Schematic of PEG hydrogels formation, (D) FTIR spectrum of 4 arm-Mal-PEG and MMP-2 sensitive PEG hydrogels.

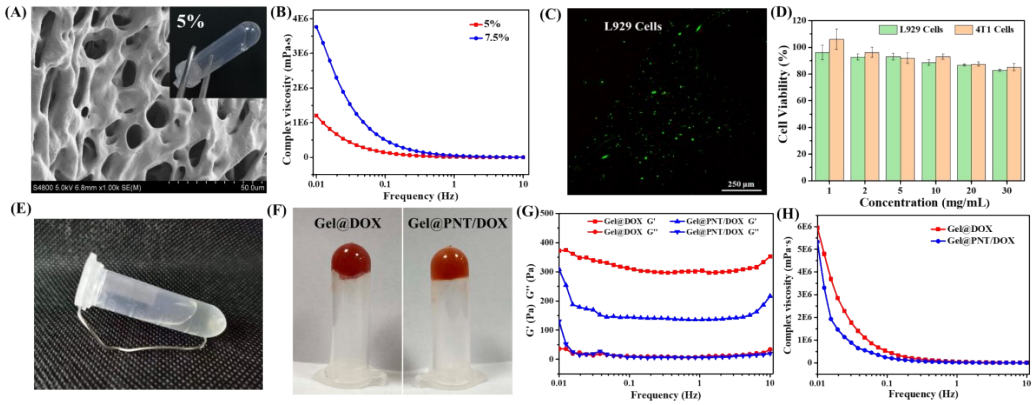

Fig. S2. (A) Morphology and SEM images of 5% PEG hydrogels. (B) Dynamic complex viscosity of MMP-2 sensitive PEG hydrogels (5% and 7%, w/v) as a function of angular frequency for the PEG hydrogels. (C) Live/dead assay of L929 cells treated with 7.5% PEG hydrogels. (D) The cytotoxicity of gelators incubated with L929 cells and 4T1 cells. (E) The image of 7.5% PEG hydrogels upon treatment with MMP-2 (200 ng/mL). (F) Morphology images of Gel@DOX and Gel@PNT/DOX. The rheological property (G) and dynamic complex viscosity (I) of Gel@DOX and Gel@PNT/DOX.

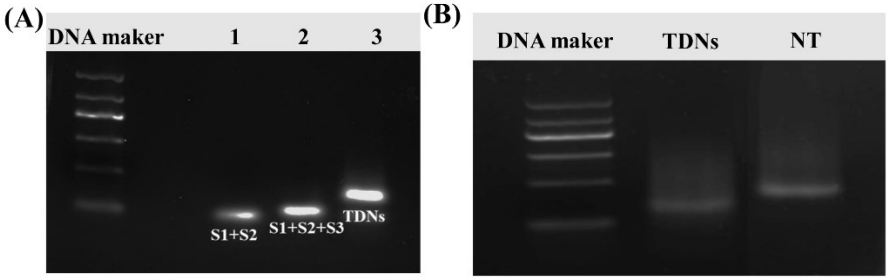

Fig. S3. (A) Gel retardation assays of TDNs. DNA marker, line 1: S1 + S2, line 2: S1 + S2 + S3, and line 3: TDNs. (B) Gel retardation assays of NT.

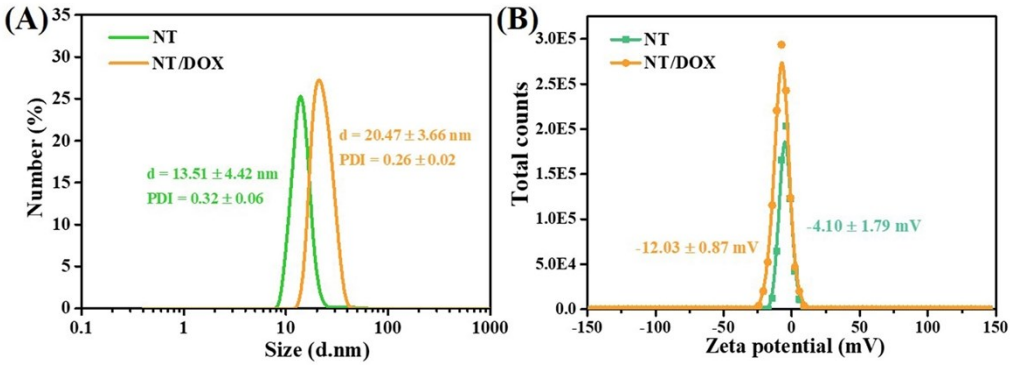

Fig. S4. The size (A) and zeta potential (B) of NT and NT/DOX

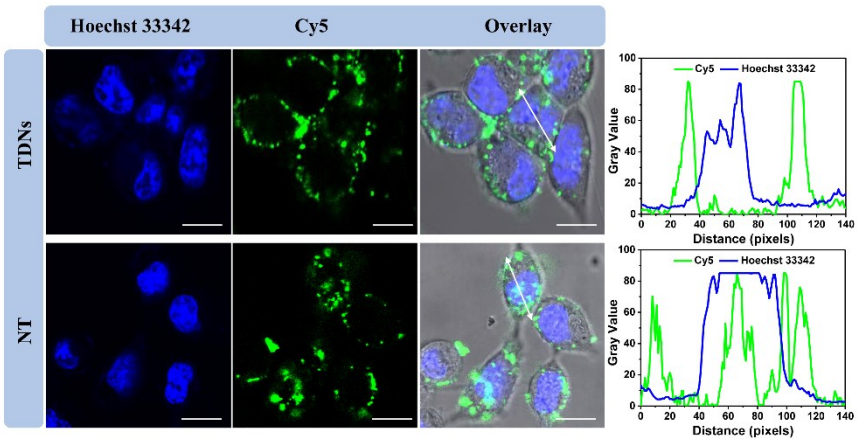

Fig. S5. The CLSM images and fluorescence intensity profile analysis of 4T1 cells treated with TDNs and NT. (TDNs was modified with Cy5, green. The scale bar represents 25 μm)

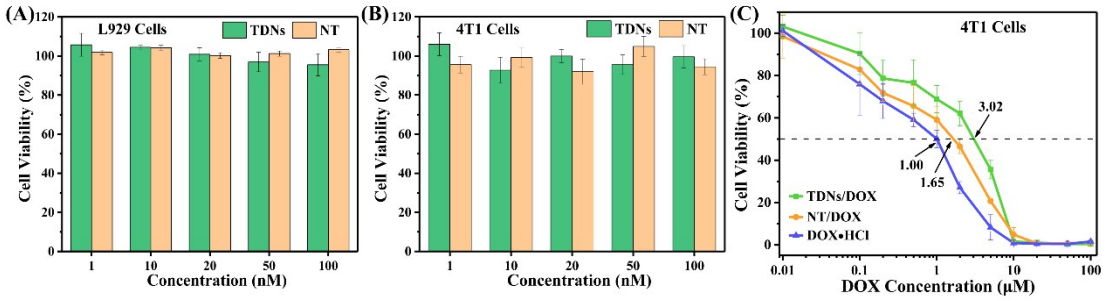

Fig. S6. *In vitro* cell viability of TDNs or NLS-TDNs incubated with L929 (A) and 4T1 (B) cells for 48 hours. The anticancer activity of free DOX, TDNs/DOX, NT/DOX against 4T1 cells (C).

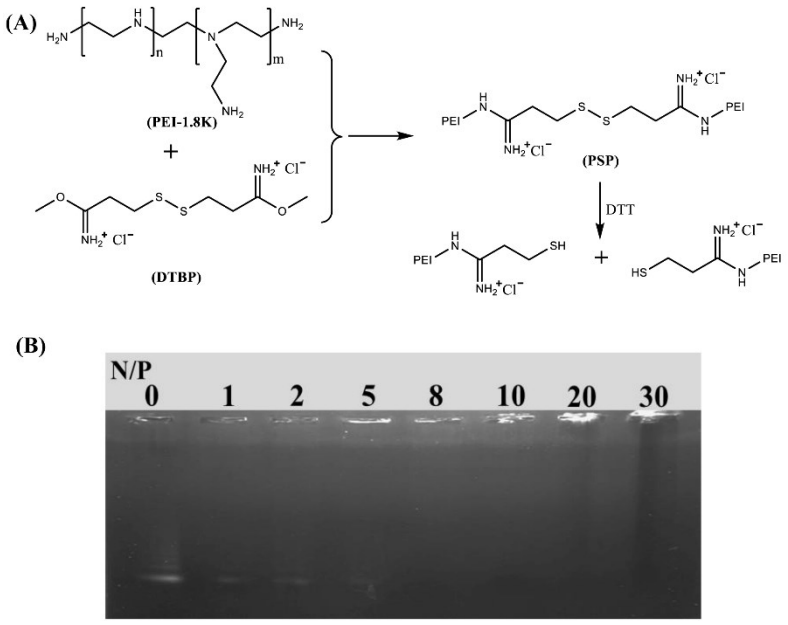

Fig. S7. (A) The synthetic route of cross-linked PEI (PSP) and the effect of disulfide bond reduction by DTT. (B) Gel retardation analysis of NT complexed with PSP at various N/P ratios.

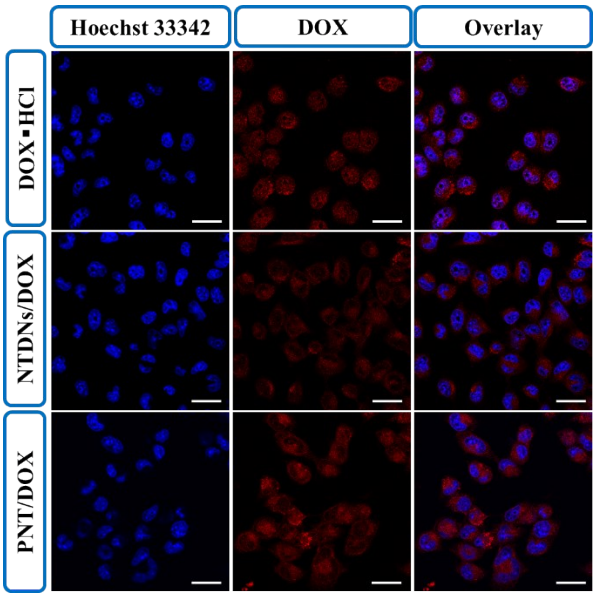

Fig. S8. The CLSM images of 4T1 cells treated with free DOX, NT/DOX, PNT/DOX for 4 h. The scale bar represented 25 μm.

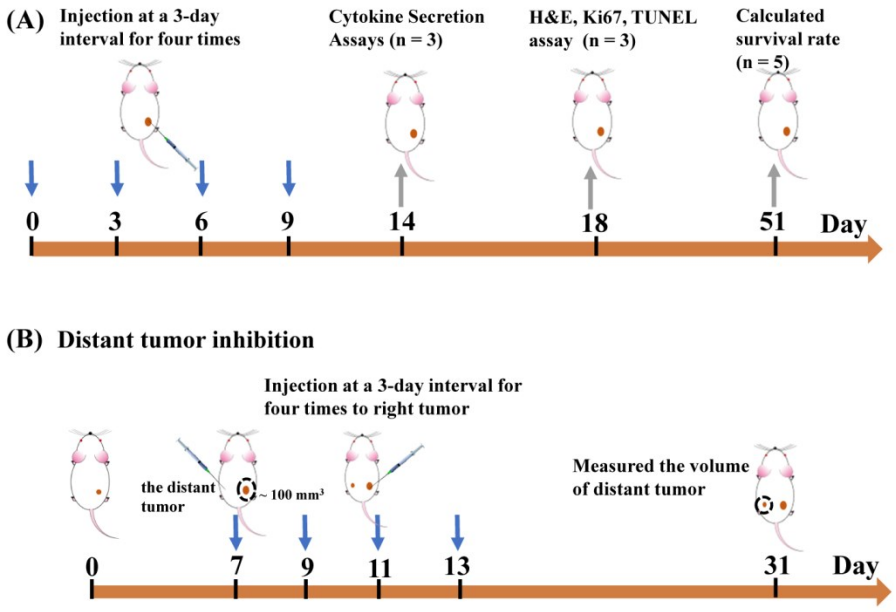

Fig. S9. Schematic illustration to show the experimental route for *in vivo* antitumor efficacy studies.

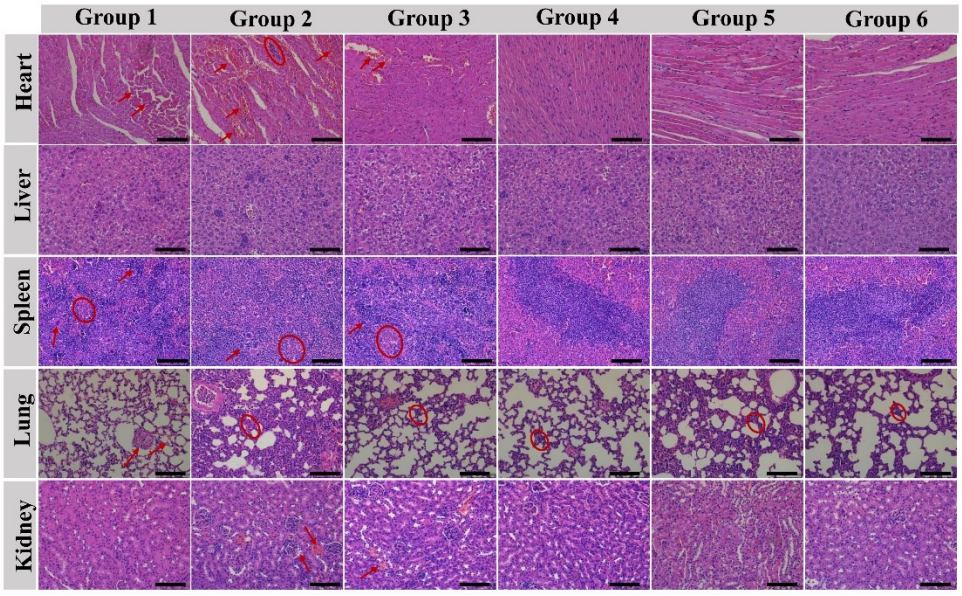

Fig. S10. Histological analysis of different organs of 4T1 tumor-bearing mice. Red circles indicated inflammation, red arrow indicated bleeding or other lesions (scale bars represent 100 μm).

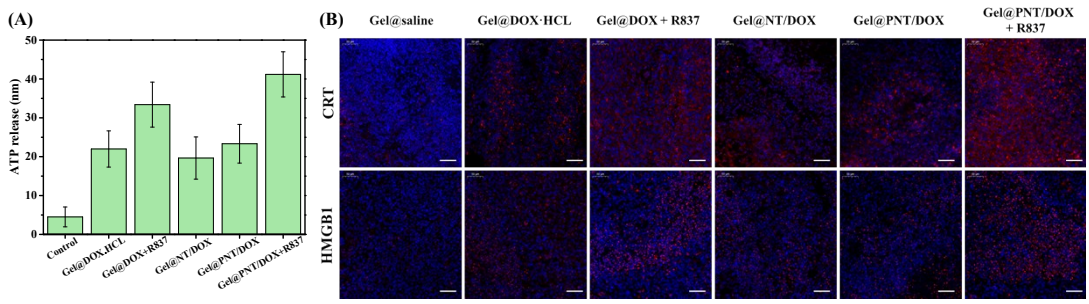

Fig. S11. (A)In vitro measurement of ATP production in 4T1 cells. (B)The CLSM images of CRT or HMGB1 staining of tumor (scale bars represent 50 μm).

Table S1. DNA oligonucleotides used in TDNs.

ssDNA	Sequence (5'-3')
S1	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
S2	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
S3	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
S4	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGG ACCCTCGCAT
S1-N_3	N_3- TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC