

Supplementary Information for

Somatostatin receptor-targeted polymeric nanoplatform for efficient CRISPR/Cas9 gene editing to enhance synergistic hepatocellular carcinoma therapy

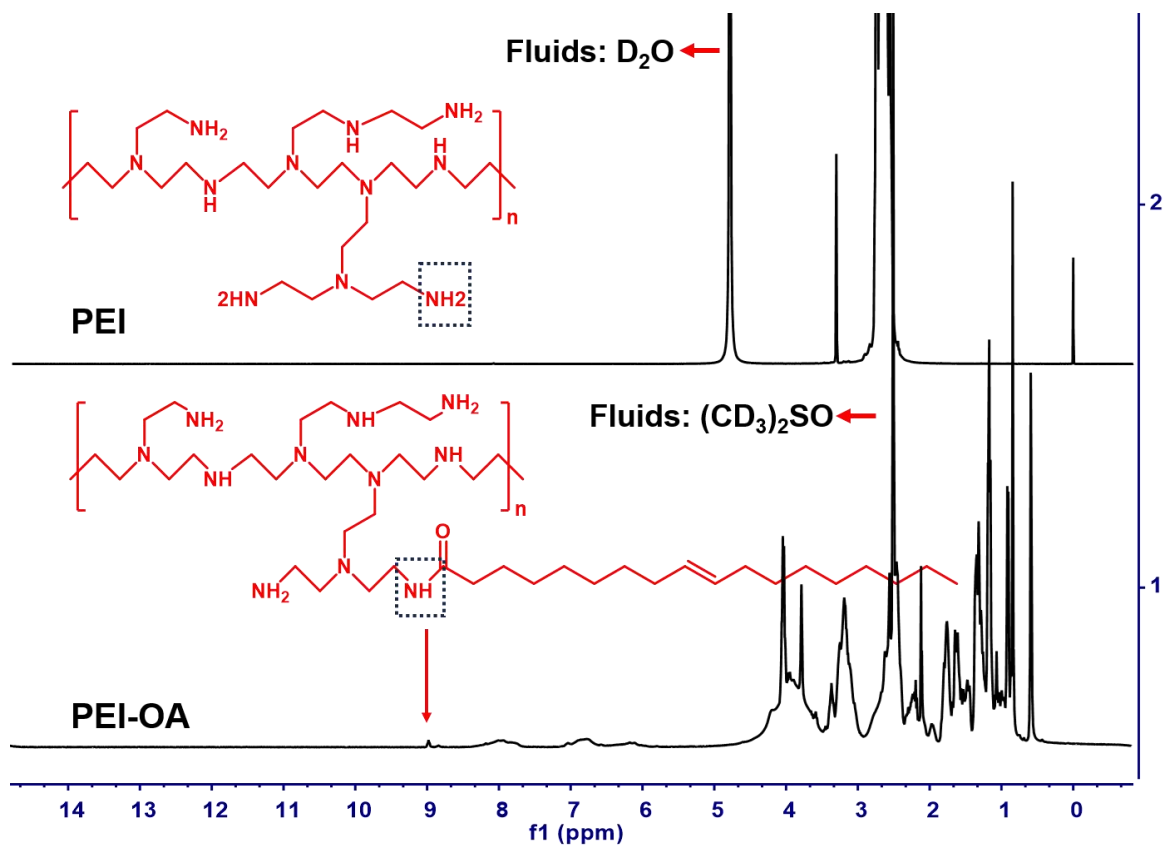
Materials

Polyethyleneimine (PEI, molecular weight 1800 Da), linoleic acid functionalized polyethylene glycol (LNA-PEG-NHS), and oleic acid (OA) were obtained from Shanghai Aladdin Biochemical Technology Co (Shanghai, China). PTX and Octreotide acetate (OCT) were purchased from Dalian Mei-lun Biotech Co., Ltd. (Dalian, China). LentiCRISPR V2 plasmid and pUC18 noload plasmid were preserved in our lab. RNA extraction Kit/Cell Total RNA Isolation Kit were purchased from FOREGENE (Chengdu, China), reverse transcription kit Fastking RT Kit were purchased from TIANGEN (Beijing china). TranscriptAid T7 High Yield Transcription Kit were purchased from Thermo Scientific (Shanghai China). The Genome-Editing Mutation Detection Kit was purchased from Beyotime (Shanghai China).

Genome sequences are queried in the official NCBI website, then the PD-L1(CD274)-targeted pCas9 plasmid (encoding Cas9 protein, as well as transcribing sgRNA 5'-GTGAAATTGCAGGATGCAG-3' that targets to PD-L1) (Fig. S1) were synthesized by the TranscriptAid T7 High Yield Transcription Kit. All primers were ordered from genecreate Co, Ltd (Wuhan, China). Human liver tumor cells (HepG2) and 293T cells were obtained from the Chinese Academy of Science Cell Bank (Shanghai, China).

Results

a)



b)

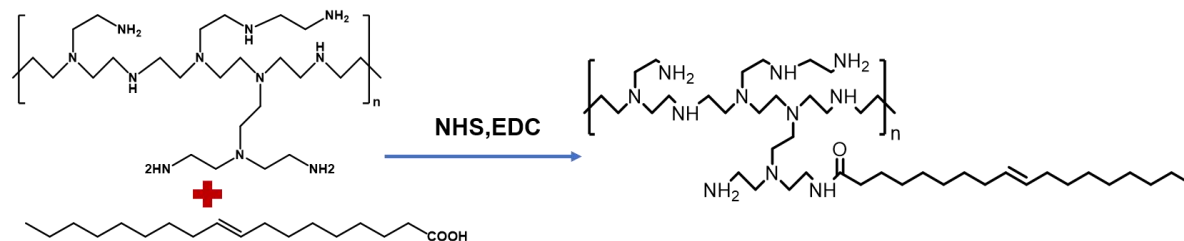


Fig.S1. a) ^1H -NMR of the PEI and PEI-OA. The solvents are D_2O and $(\text{CD}_3)_2\text{SO}$. One H atom in the $-\text{NH}_2$ group of PEI was replaced by OA, resulting in a characteristic peak appearing in the spectrum at δ 8.97 ppm. b) Synthesis process of PEI-OA.

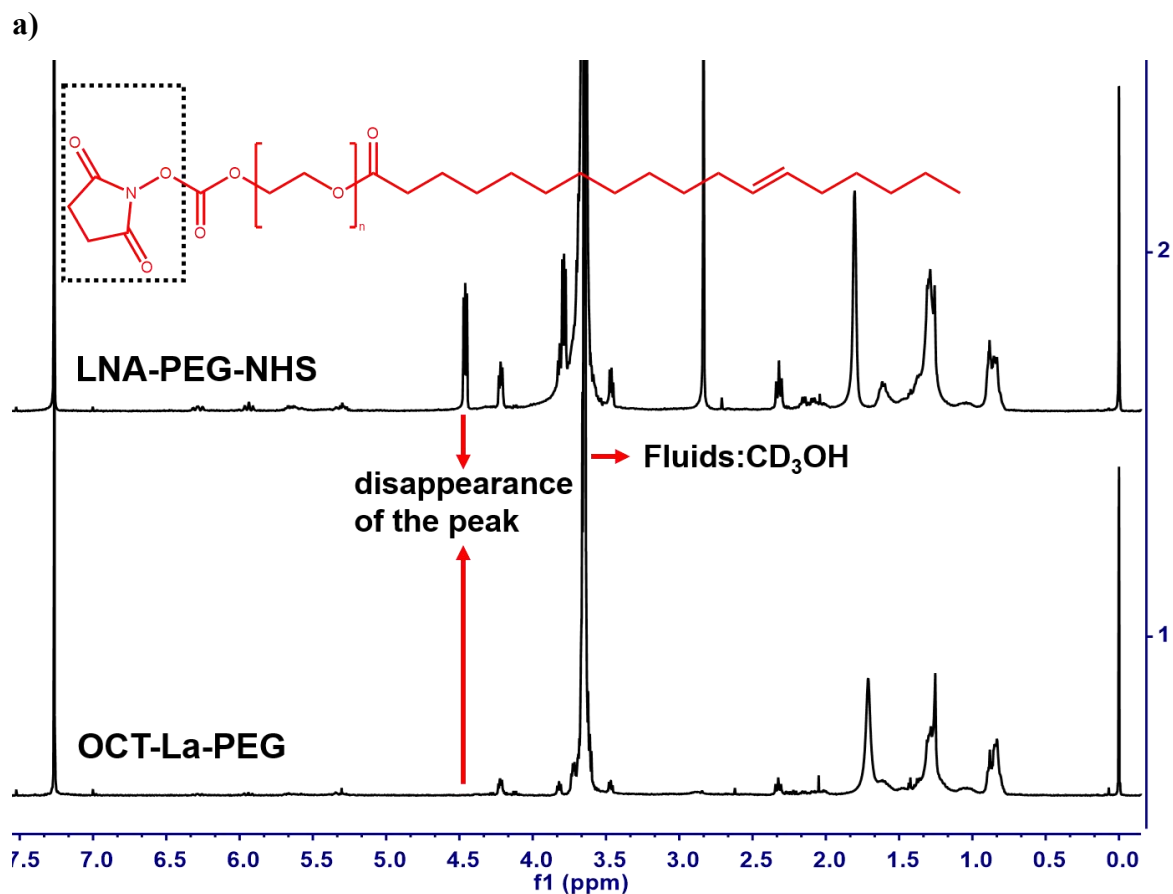
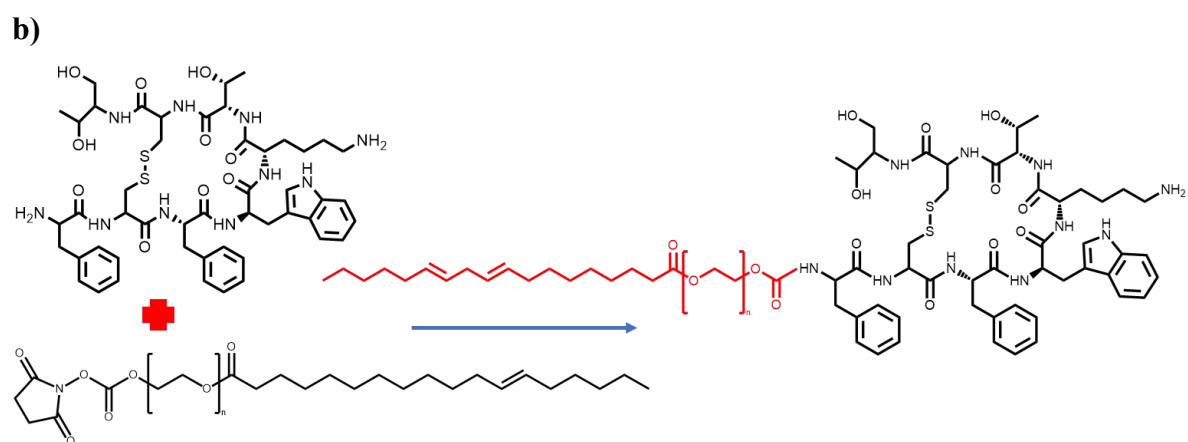


Fig.S2. a) ^1H -NMR of the LNA-PEG-NHS and LNA-PEG-OCT. The solvent is CD_3OD . The δ 7.25 ppm peak is characteristic of LNA-PEG-NHS, and the disappearance of the peak (δ 4.45 ppm) in the ^1H -NMR results of LNA-PEG-OCT proves the successful coupling of octreotide to LNA-PEG-NHS. b) Synthesis process of LNA-PEG-OCT.

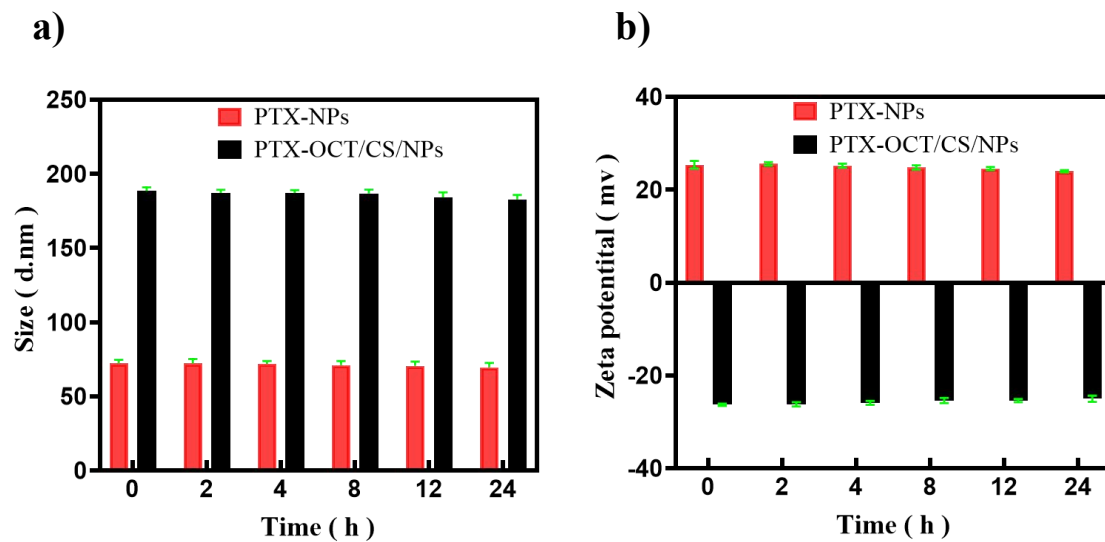


Fig.S3. **a)** The particle size of nanoparticles in the serum stability experiment. **b)** The charge of nanoparticles in the serum stability experiment.

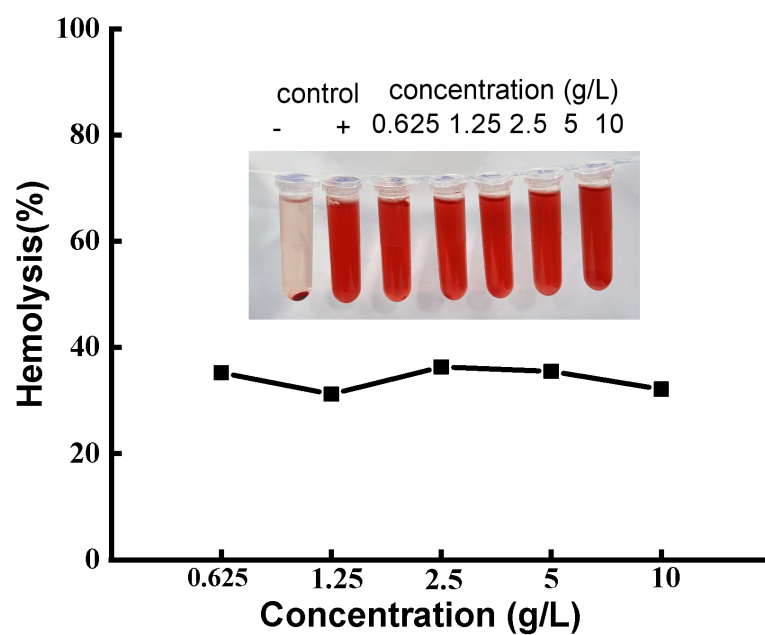


Fig.S4. The hemolytic activity of the NPs without CS.

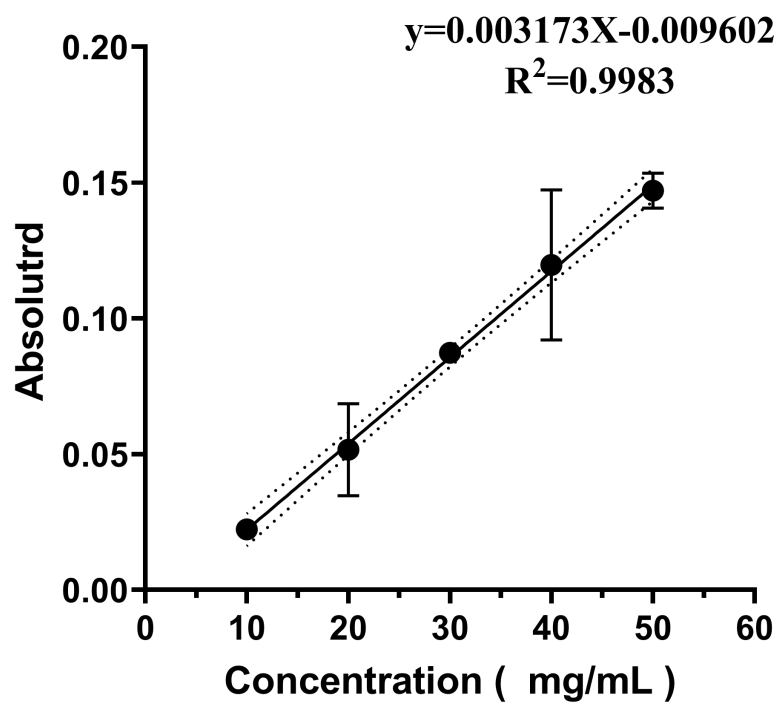


Fig.S5. Standard curve of PTX for in vitro release assay.

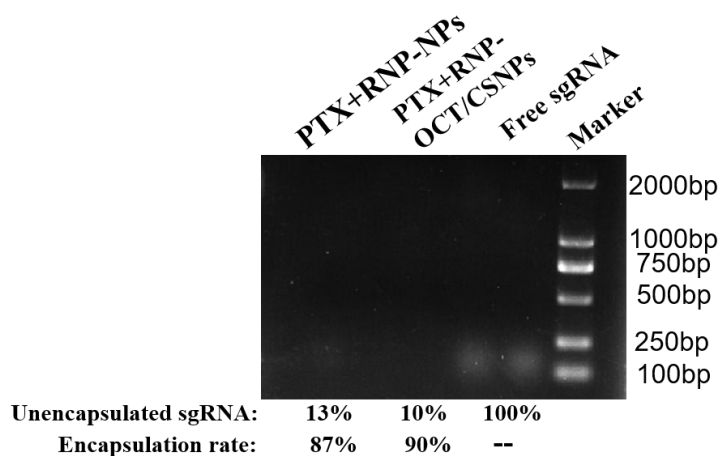


Fig.S6. Gel retardation assay for detecting the encapsulation efficiency of RNP.

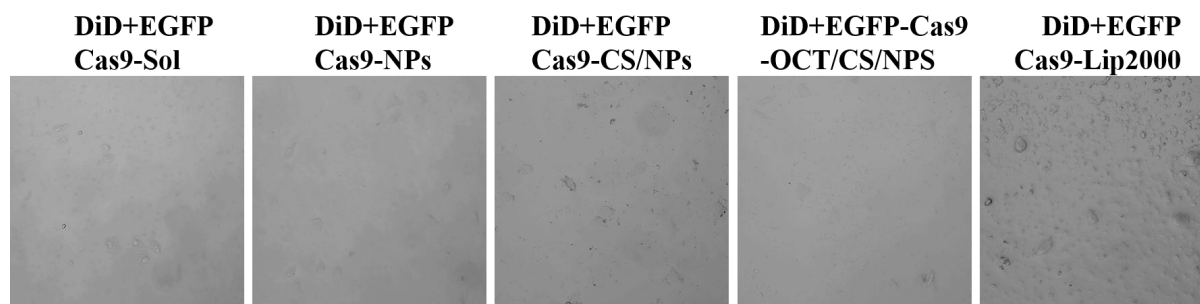


Fig.S7. Enlargement of image 3a bright in the main text.

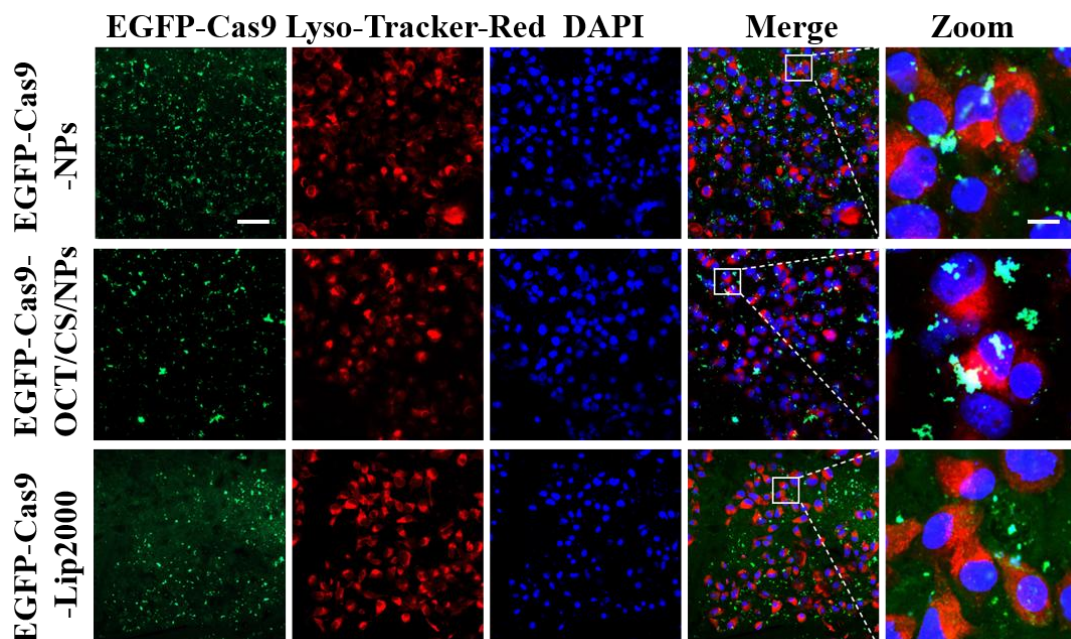


Fig.S8. Lysosomal immune escape assay after treatment of EGFP-Cas9 in different groups
Scale bar, 100 nm.

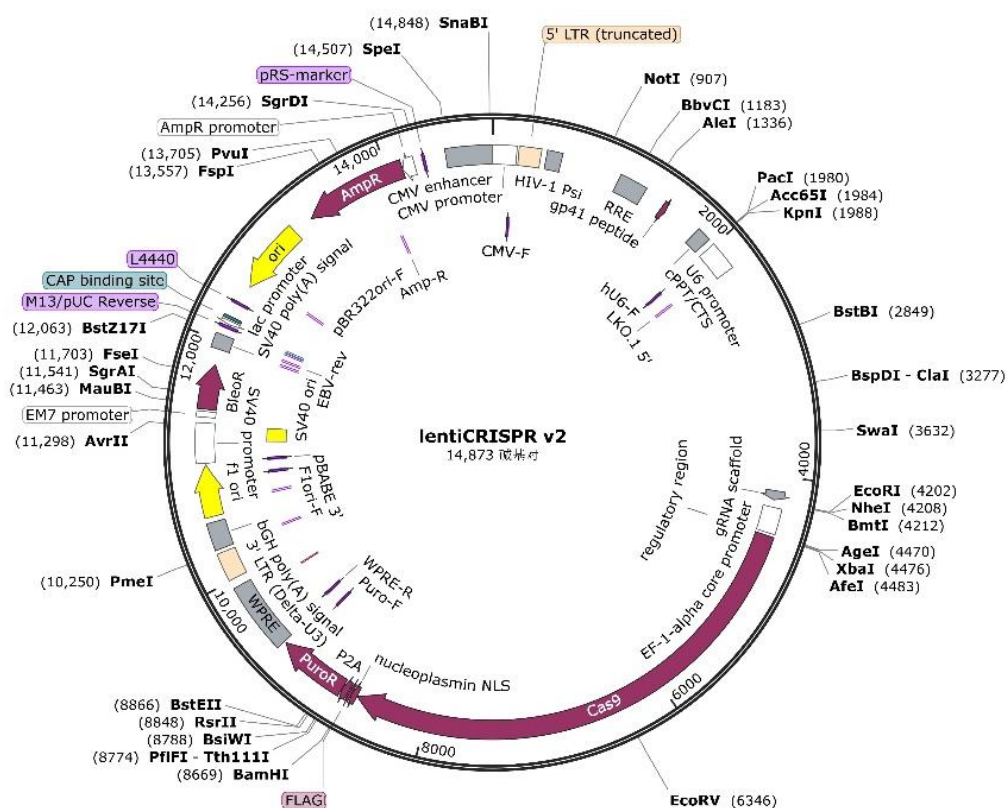


Fig.S9. Plasmid map of LentiCRISPR V2 DNA vector.

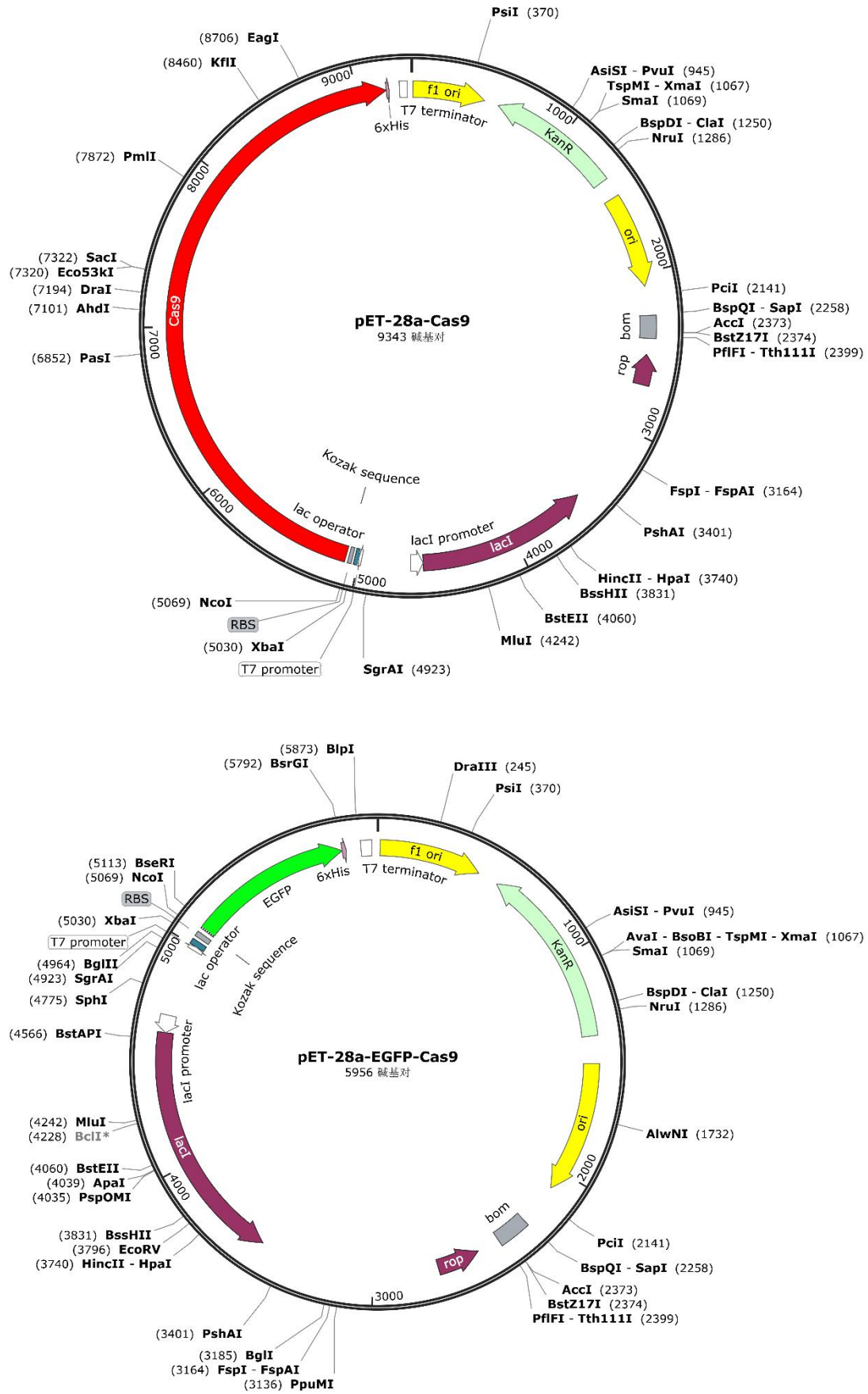


Fig.S10. Plasmid map of pET-28a-Cas9 (above) and pET-28a-Cas9-EGFP (below).

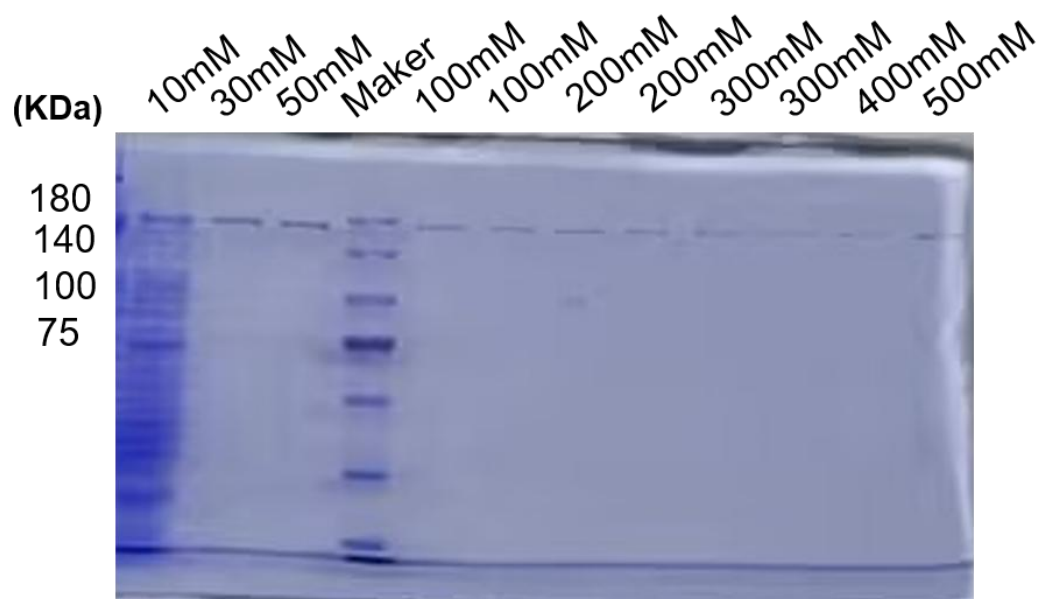


Fig.S11. Purification process and elution gradient of Cas9 protein and Cas9-EGFP protein.

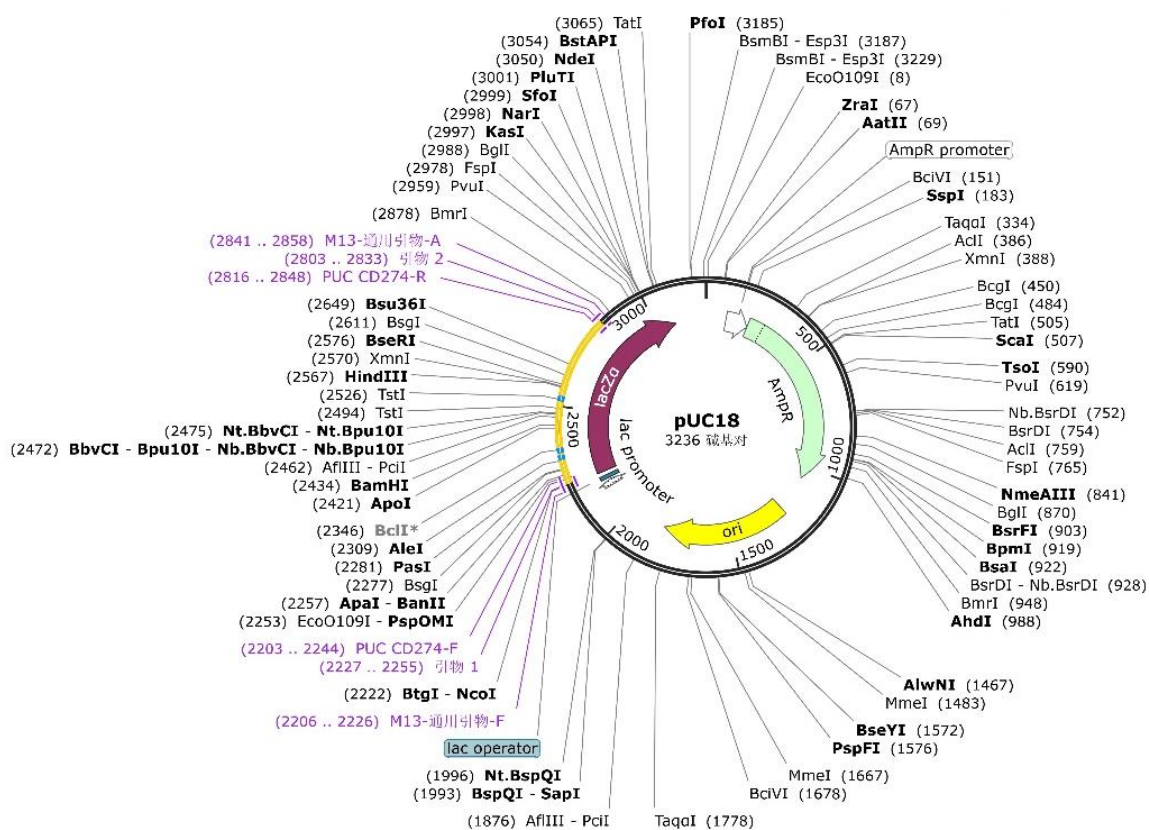


Fig.S12. Plasmid map of PUC18-PD-L1 DNA vector.

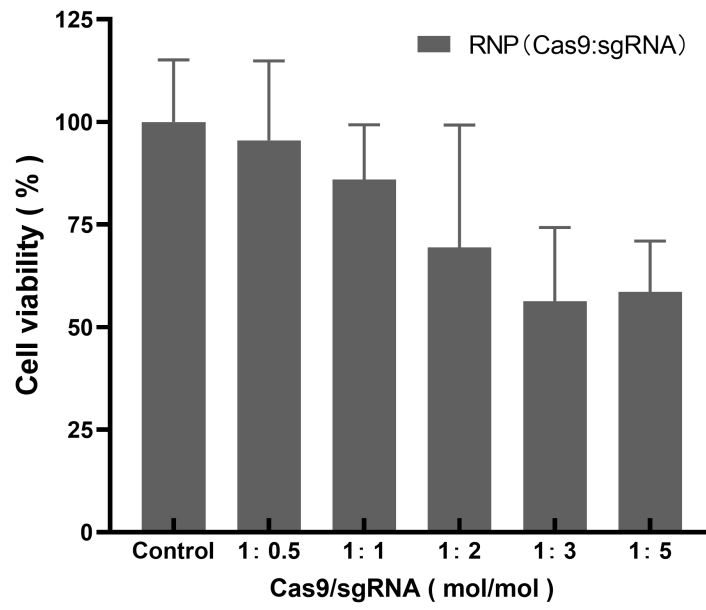


Fig.S13. MTT assay to verify the optimal ratio of RNP (Cas9:sgRNA).

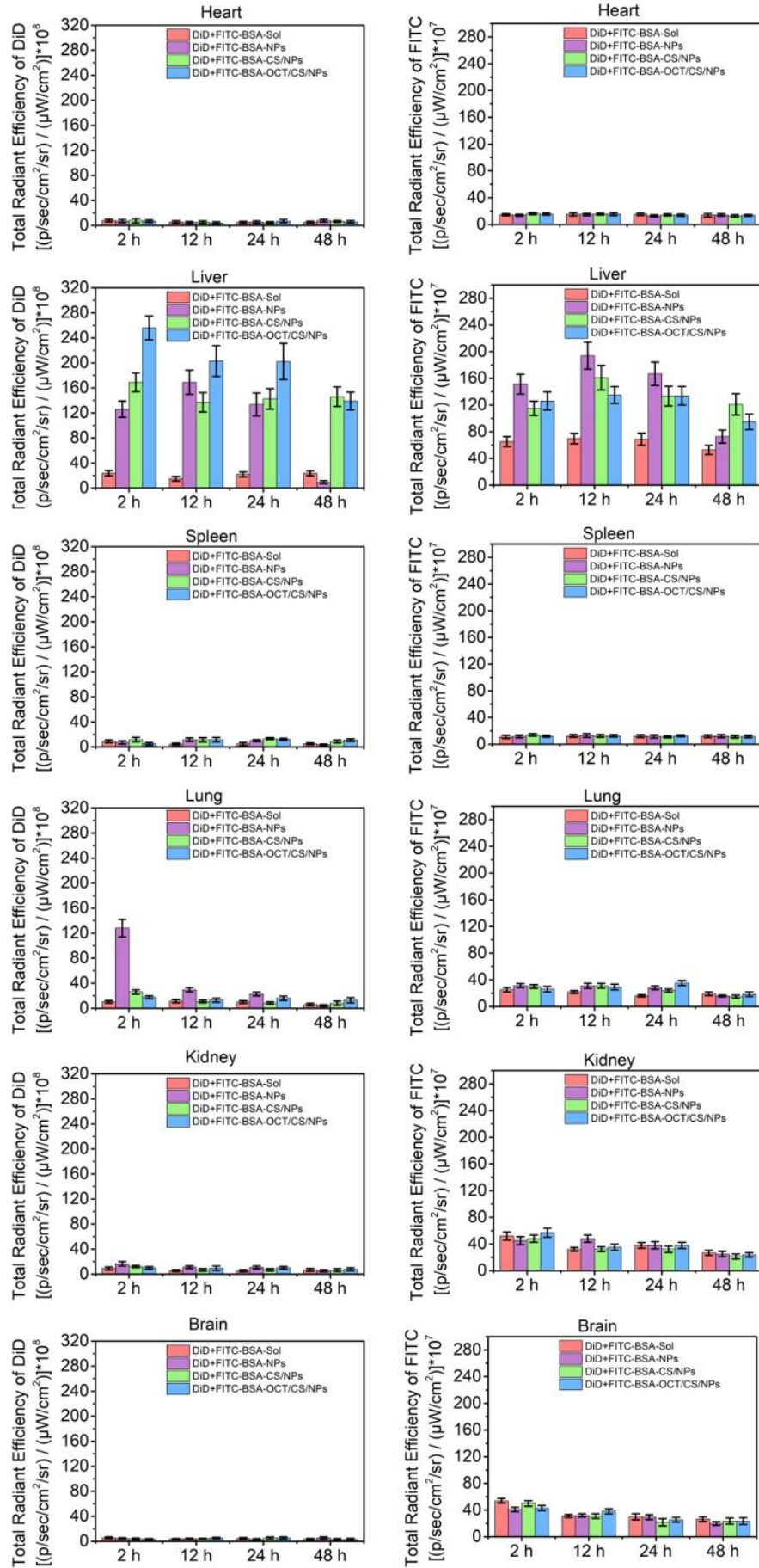


Fig.S14. Fluorescence quantification of isolated tissues.

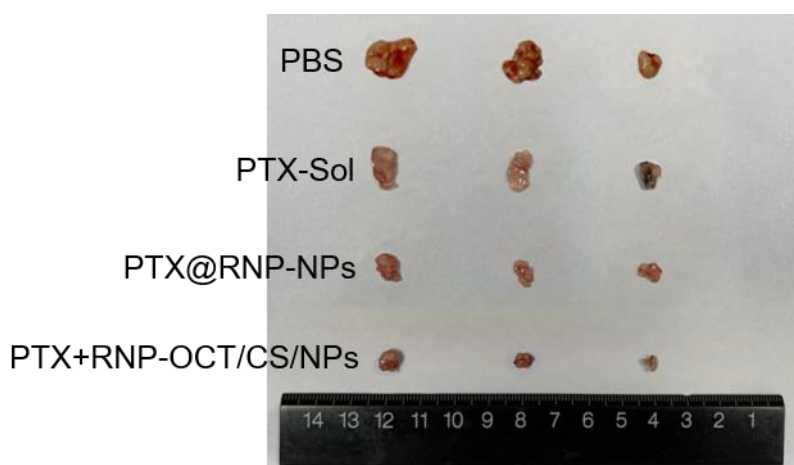


Fig.S15. Pictures of dissected mouse tumors at the end of treatment.

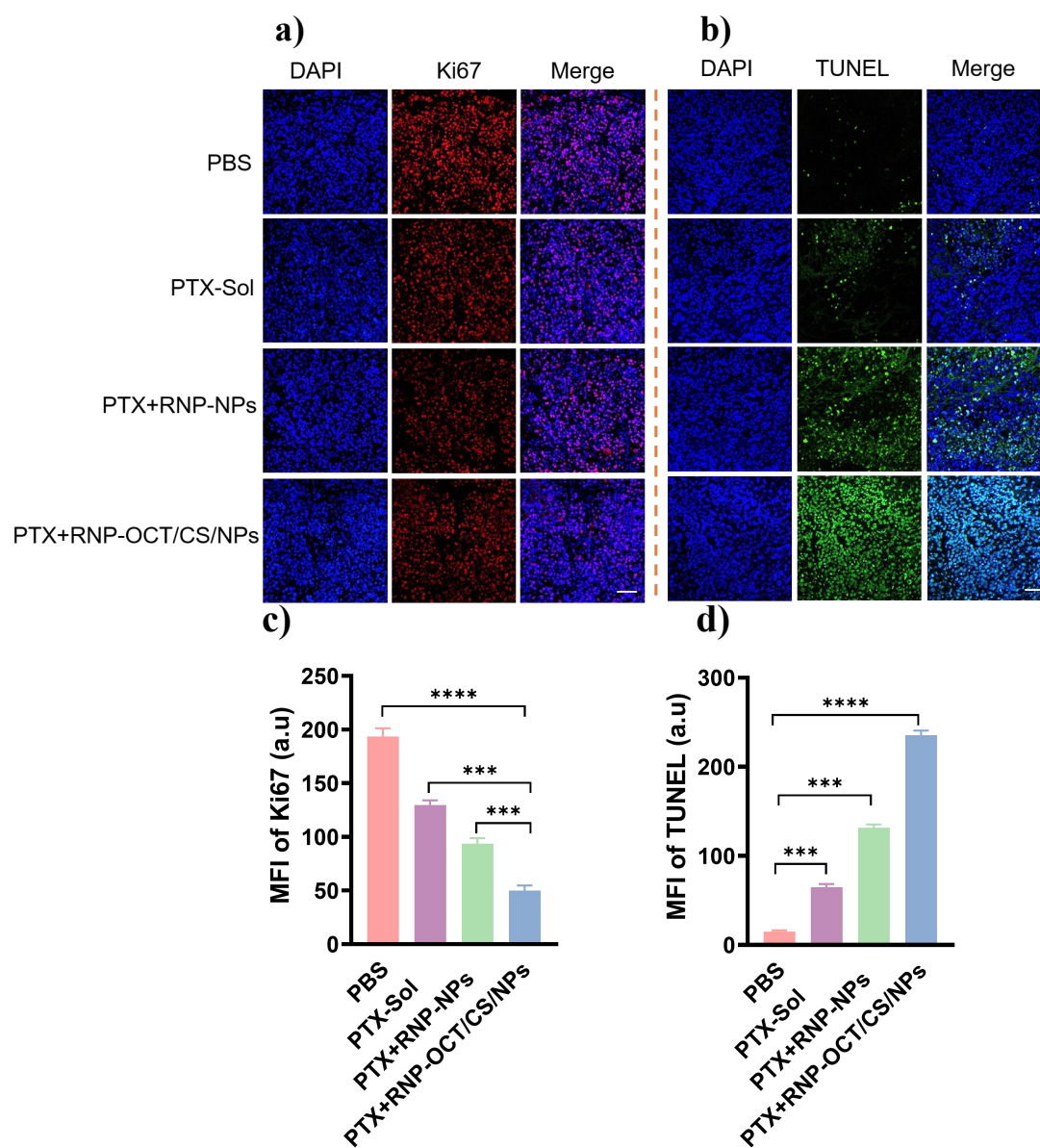


Fig.S16. a) Immunofluorescence of tumor tissue stained with Ki67 at the end of treatment. b) Immunofluorescence of tumor tissue stained with TUNEL at the end of treatment. c) Mean fluorescent intensity (MFI) of the Ki-67 signal. d) MFI of TUNEL signal.

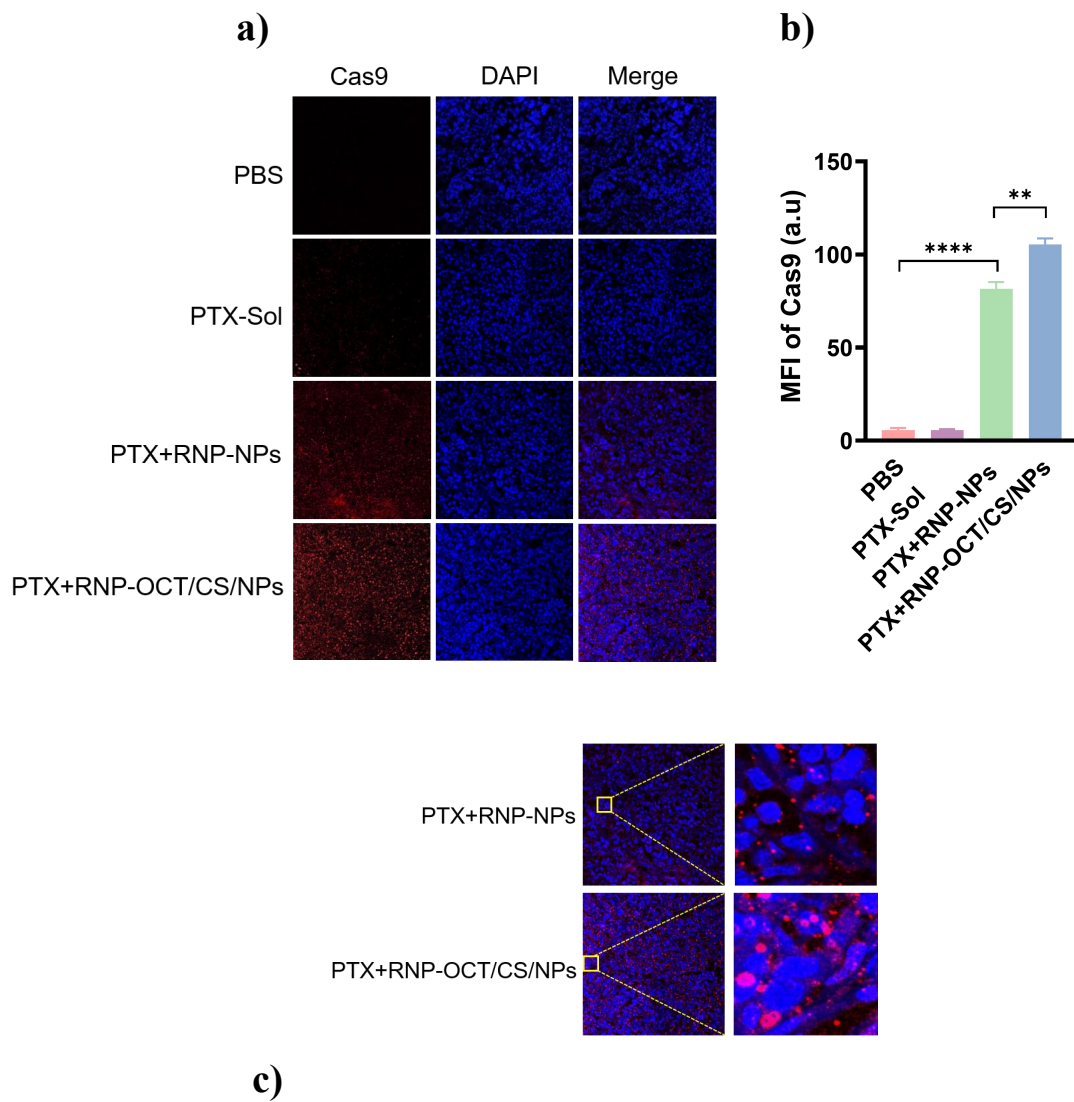


Fig.S17. a) Immunofluorescence of tumor tissue stained with Cas9 at the end of treatment. b) Mean fluorescent intensity (MFI) of the Cas9 signal. c) The 10x magnification of merge.

Table.S1. Sequences of sgRNA primers.

Target locus	Sequence	Primer F	Primer R
sgPD-L1-1	GTGAAATTGC AGGATGCAG	TTCTAATACGACTCACTATA GGTGAAATTGCAGGATGC AGGTTTTAGAGCTAGAAAT	GCACCG ACTCG
sgPD-L1-2	TACCGCTGCA TGATCAGCTA	TTCTAATACGACTCACTATA GTACCGCTGCATGATCAGC TAGTTTTAGAGCTAGAAAT	GCACCG ACTCG
sgGFP	CAAGATCCGC CACAAACATCG	TTCTAATACGACTCACTATA GCAAGATCCGCCACAACAT CGGTTTTAGAGCTAGAAAT	GCACCG ACTCG

Table.S2. Sequences of DNA oligos

Target locus	Primer F	Primer R
PD-L1	GGTTCAGCATAGTAGCTACAGA CAGAGGG	TGTAGCTTGGATGCCACAT TTTTTCACATCC
Cas9	TATACCATGGGCAGCAGCATGG ACAAGAAGTACAGCATCGG	GTCTCAGCTGGGAGGCGA CCACCACCACCACCACCA C
pET28a	CACCACCACCACCACCACTGA GAT	AGGAGATATACCATGGGCA GCAGC
Puc18	TAGCTACTATGCTGAACCCATG GTCATAGCTGTTTCCTGTGT	TGTGGCATCCAAGATACAA TTACGGCACTGGCC