Supporting Information

Appropriate Delivery of the CRISPR/Cas9 System through the Nonlysosomal Route: Application for Therapeutic Gene Editing

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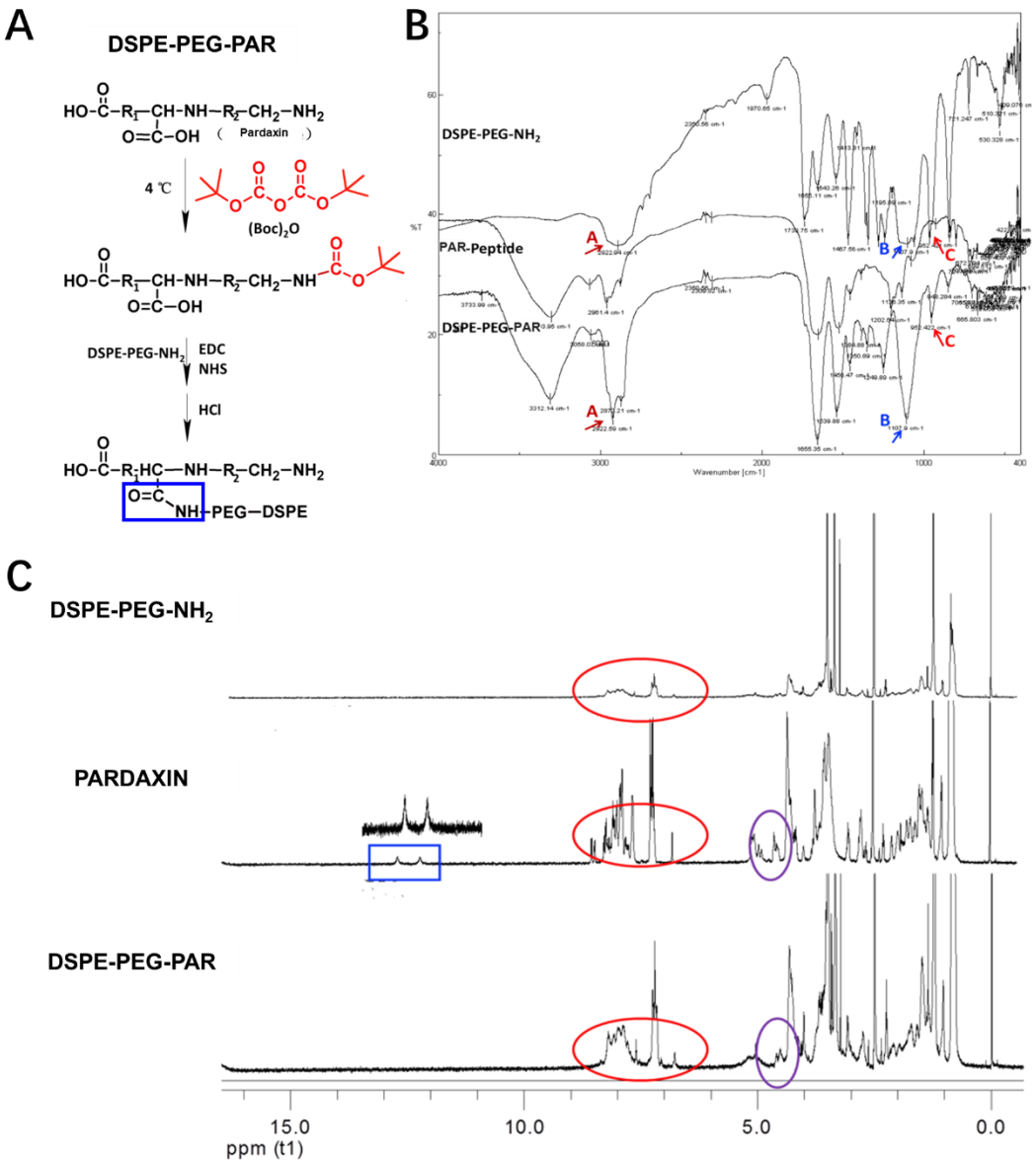


Figure S1. Synthesis and characterization of DSPE-PEG-PAR. (A) Organic synthetic route of DSPE-PEG-PAR. (B) The FT-IR spectrum of DSPE-PEG-NH_2, PARDAXIN, and DSPE-PEG-PAR, (A, ν C-H=2922.59 cm^{-1} B, ν C-O=1107.9 cm^{-1}, C, Δ C-H (-CH_2-

)=952.422 cm^{-1}) . (C) The ^{1}H-NMR (400 MHz, DMSO-d_6) of DSPE-PEG-NH_2, PARDAXIN, and DSPE-PEG-PAR. The signal in (C) corresponds to the structure in (A).

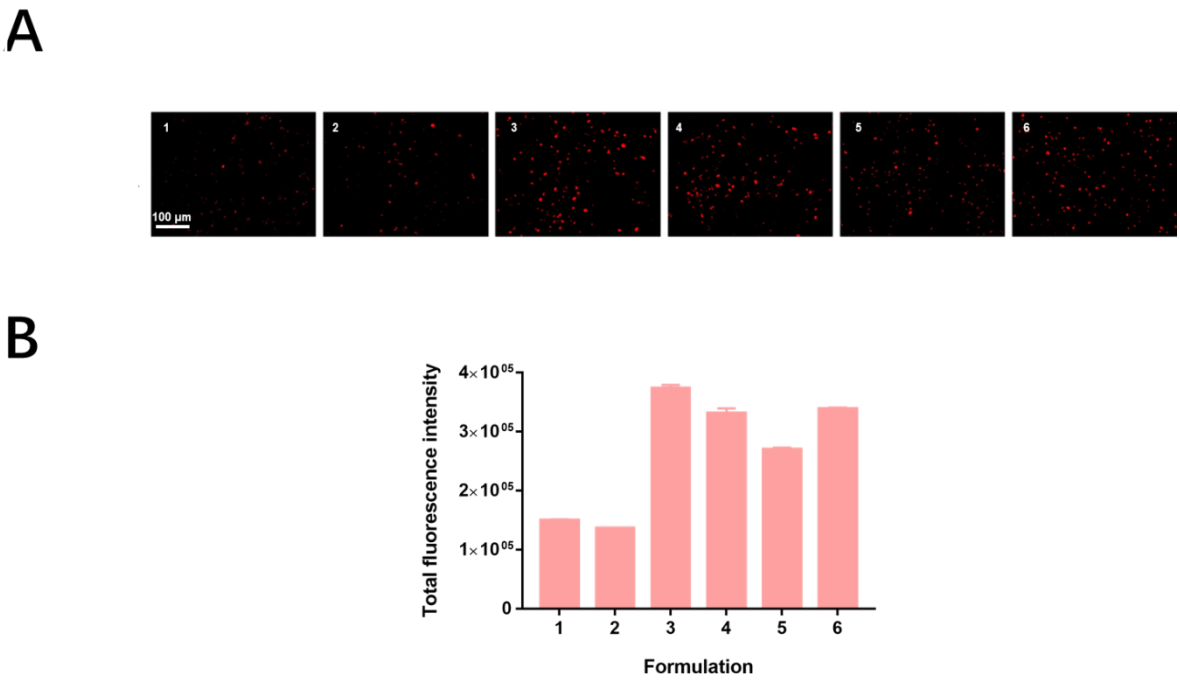


Figure S2. Screening of optimal prescriptions for transfection. (A) The cellular uptake efficiency of different Pardaxin-modified cationic liposome/DNA (Cy3 labeled) complexes (weight ratio: 5:1) and the total fluorescence intensity (B).

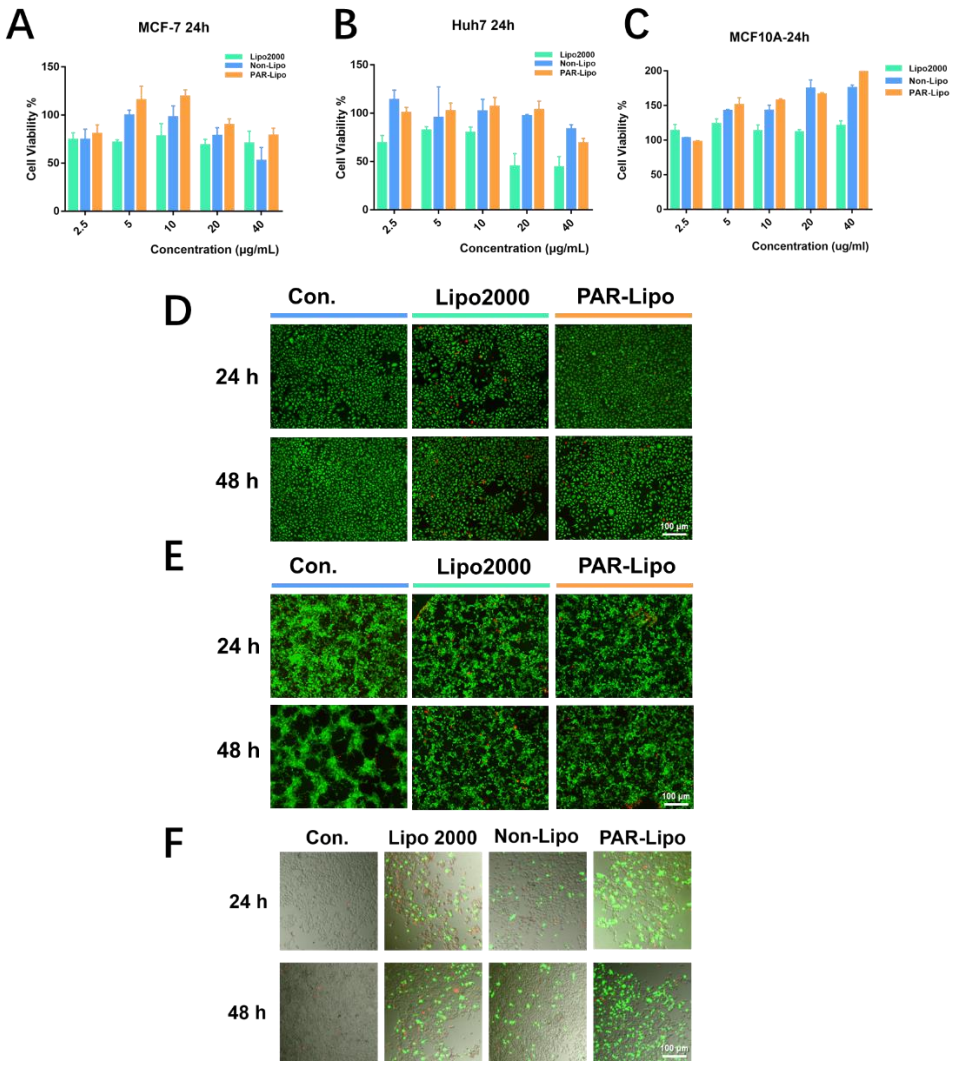
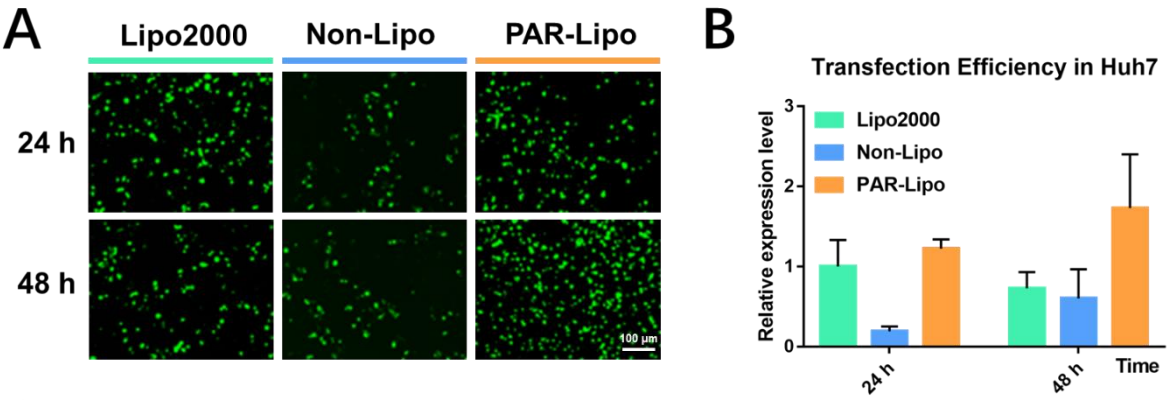
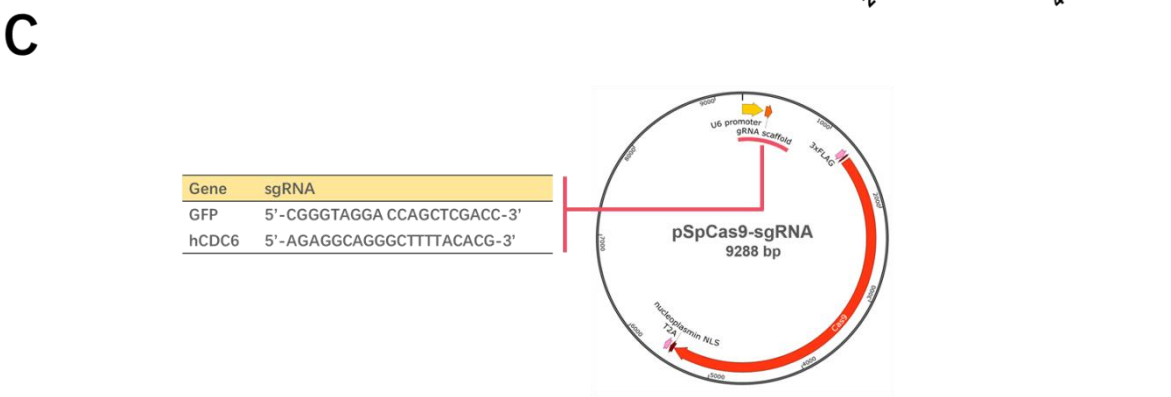
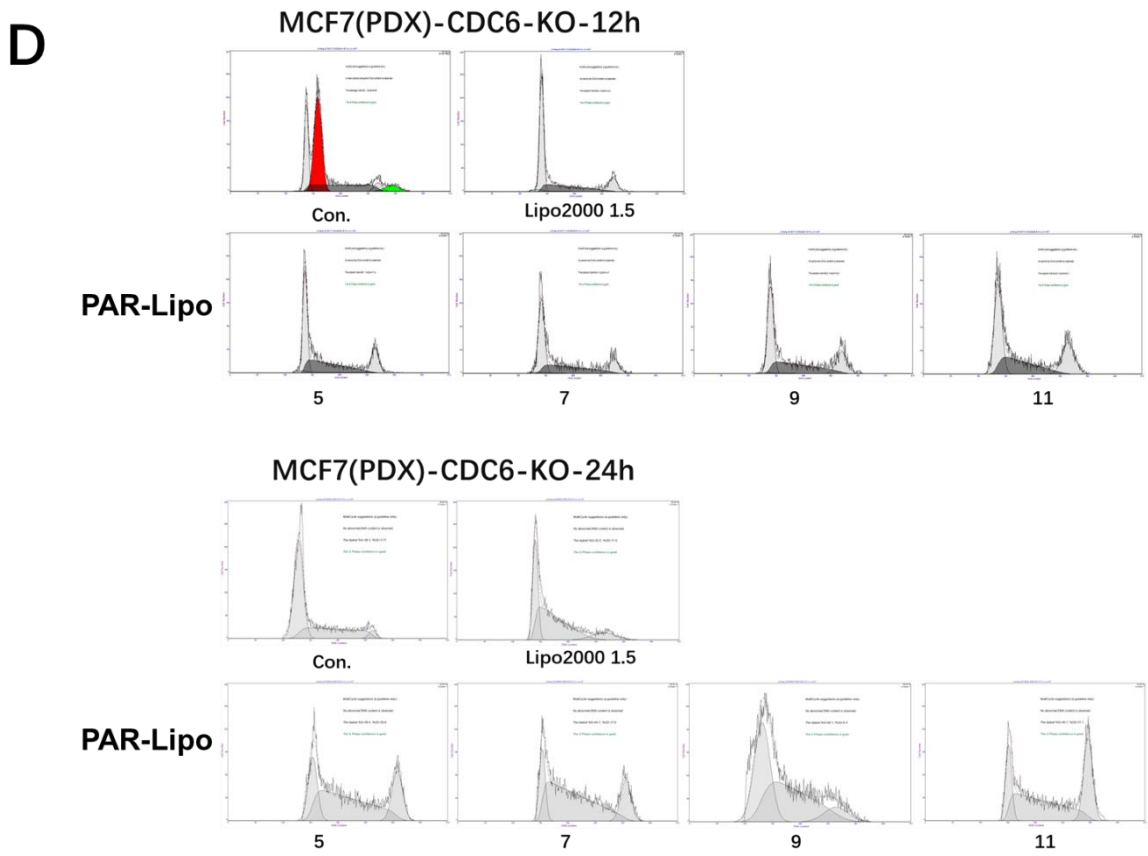


Figure S3. Cytotoxicity. Cytotoxicity of cationic liposomes to MCF-7 (A) , Huh7 (B) and MCF10A (C). The cytotoxicity of normal cells, LO2 (D) and HEK293T (E) was investigated using a live/dead staining method. Transfection cytotoxicity of cationic liposomes was evaluated by Propidium Iodide (PI) staining assay (F).


A
Lipo2000
Non-Lipo
PAR-Lipo
24 h
48 h
100 μm
B
Transfection Efficiency in Huh7
Relative expression level
Lipo2000
Non-Lipo
PAR-Lipo
24 h
48 h
Time


C
Gene sgRNA
GFP 5'-CGGGTAGGA CCAGCTCGACC-3'
hCDC6 5'-AGAGGCAGGGCTTTTACACG-3'
U6 promoter
gRNA scaffold
3xFLAG
pSpCas9-sgRNA
9288 bp
nucleoplasmin NLS
T2A


D
MCF7(PDX)-CDC6-KO-12h
Con.
Lipo2000 1.5
PAR-Lipo
5
7
9
11
MCF7(PDX)-CDC6-KO-24h
Con.
Lipo2000 1.5
PAR-Lipo
5
7
9
11

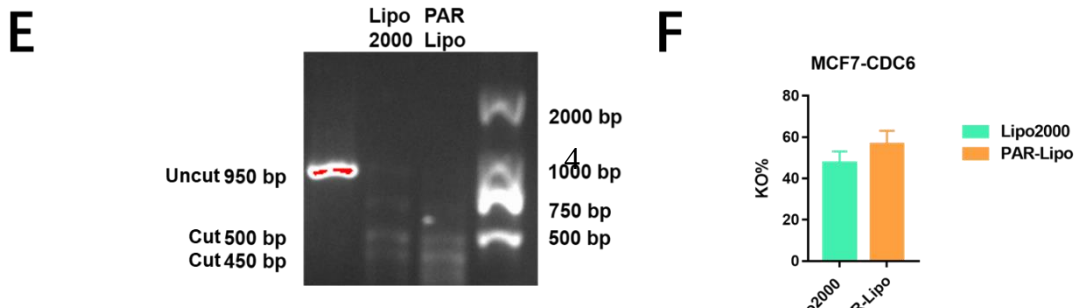

E
Lipo 2000
PAR Lipo
Uncut 950 bp
Cut 500 bp
Cut 450 bp
2000 bp
1000 bp
750 bp
500 bp
F
MCF7-CDC6
KO%
Lipo2000
PAR-Lipo

Figure S4. The capacity of transfection in Huh7. (A) Green fluorescence proteins expression 24 h and 48 h after transfection by three cationic liposomes/pEGFP complexes to Huh7 cells. (B) The transfection efficiency semiquantitative analysis by ImageJ. (C) The plasmid was used to delivery CRISPR/Cas9 gene editing system. (D) The flow cytometry analysis images of MCF7-CDC6-KO. (E) Agarose gel electrophoresis results via T7E1 assay after 72 h transfection by Lipo2000/plasmid complexes (1.5:1) and PAR-Lipo/plasmid complexes (5:1). (F) Quantitative assay of agarose gel electrophoresis results using Imaging J software.

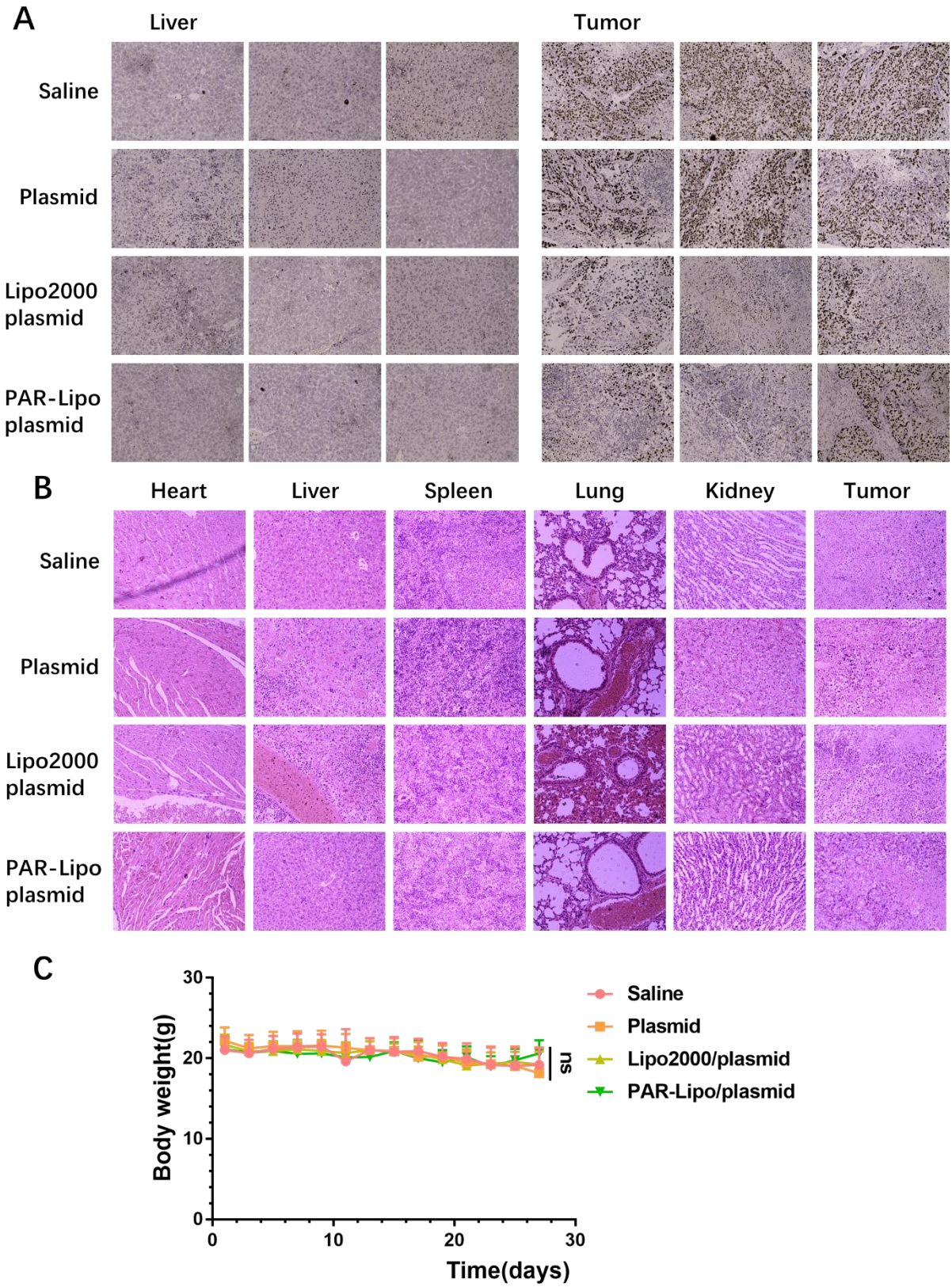


Figure S5. The safety of liposomes in MCF-7 tumor. Results of Ki67 (A) slices of tumor and liver in four groups of mice And H&E (B) slices of heart, liver, spleen, lung, kidney, tumor. (C) The changes of body weight during the treatment (ns means no statistical significance).

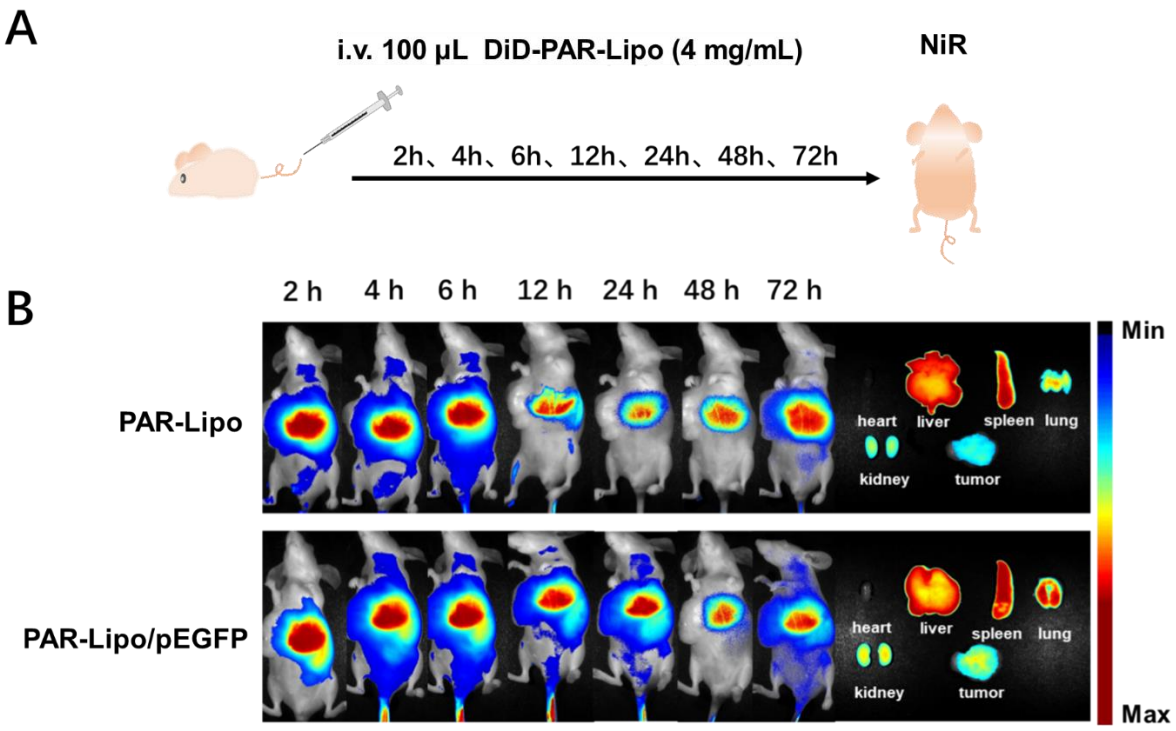


Figure S6. The distribution of PAR-Lipo and PAR-Lipo/DNA complexes *in vivo*. (A) Illustration of the liposomes administration scheme. (B) The bioluminescence images were obtained by IVIS spectrum imaging system.

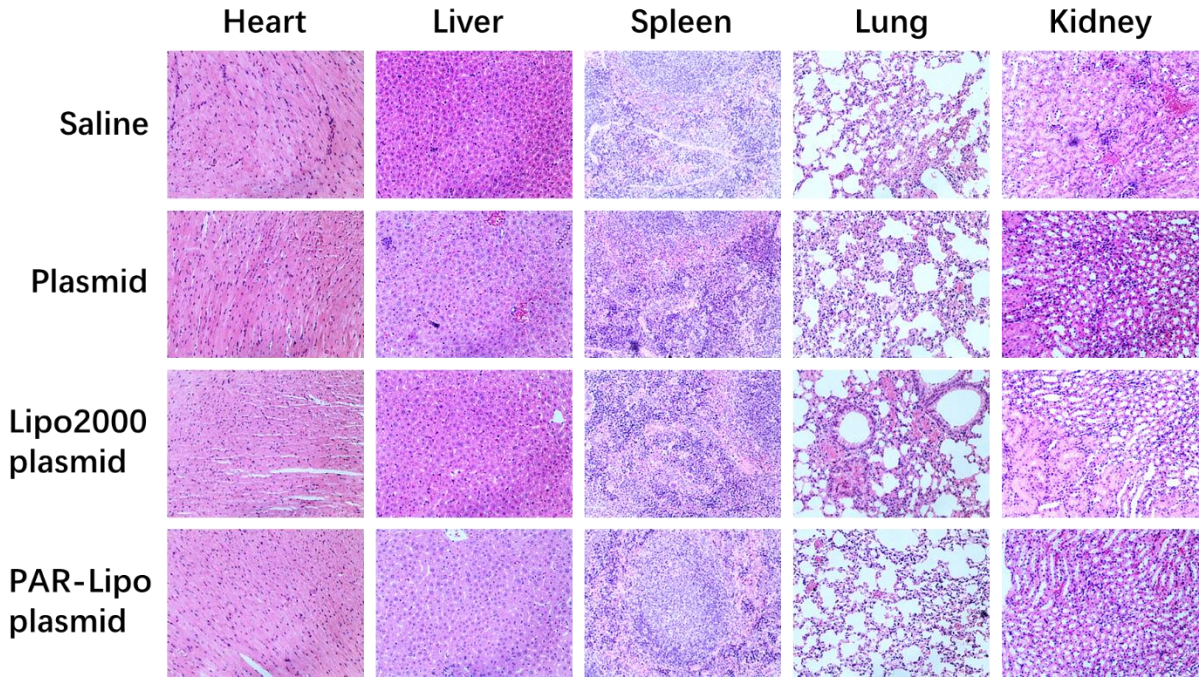


Figure S7. The safety of liposomes in Luci-Huh7 tumor. Results of H&E slices of heart, liver, spleen, lung, kidney, tumor.

Table S1. Six formulations of cationic liposomes modified with PAR peptide

Lipids (wt/wt)	Formulation					
	1	2	3	4	5	6

DOTAP : DOPE	2:1	2:1	4:1	4:1	8:1	8:1
DSPE-PEG-NH2 : DSPE-PEG-PAR	1:3	2:2	1:3	2:2	1:3	2:2

Table S2. Particle size and PDI (Polymer Dispersity Index) of PAR peptide modified liposomes and their DNA combined complexes

liposome : plasmid (wt/wt)		Formulation					
		1	2	3	4	5	6
liposome only	size (nm)	186.67	122	126.33	116	124.33	119.33
	PDI	0.219	0.35	0.373	0.400	0.368	0.386
5 : 1	size (nm)	219.33	135.33	140	133.67	165.33	245.67
	PDI	0.29	0.44	0.424	0.42	0.329	0.240