Supporting Information for

ORIGINAL ARTICLE

A combination of LightOn gene expression system and tumor microenvironment-responsive nanoparticle delivery system for targeted breast cancer therapy

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1. Materials and Methods

1.1 Synthesis of PEI-SS-VES

1.1.1 Synthesis of Boc-SS-OH

2-Hydroxyethyl disulfide (1.54 g) and DMAP (305 mg) were dissolved in 80 mL anhydrous CH_2Cl_2. Boc_2O (500 mg) dissolved in 20 mL CH_2Cl_2 was added dropwise into the solution and the reaction was stirred for 12 h under ice bath. Then the mixture was washed with saturated sodium bicarbonate solution. The organic layer was extracted and then washed with brine, dried over anhydrous Na_2SO_4, and then concentrated. The crude product was purified with silica gel column chromatography to obtain Boc-SS-OH. The chemical structure was confirmed by ^1H NMR (400 MHz, $CDCl_3$).

1.1.2 Synthesis of Boc-SS-VES

Boc-SS-OH (300 mg), EDC (226 mg) and DMAP (144 mg) were dissolved in 10 mL anhydrous CH_2Cl_2. VES (450 mg) dissolved in 10 mL CH_2Cl_2 was added dropwise into the solution and the reaction was stirred for 12 h. The crude product was purified with silica gel column chromatography to obtain Boc-SS-VES. The chemical structure was confirmed by ^1H NMR (400 MHz, $CDCl_3$).

1.1.3 Synthesis of VES-SS-OH

Boc-SS-VES (600 mg) was dissolved in 5 mL anhydrous CH_2Cl_2. 3 mL CF_3COOH was added dropwise into the solution under -45 ℃ and the reaction was stirred at -30 ℃ for 6 h. Then the mixture was washed with saturated sodium bicarbonate solution. The organic layer was extracted and then washed with brine, dried over anhydrous Na_2SO_4, and then concentrated. The crude product was purified with silica gel column chromatography to obtain VES-SS-OH. The chemical structure was confirmed by ^1H NMR (400 MHz, $CDCl_3$).

1.1.4 Synthesis of VES-SS-COOH

VES-SS-OH (300 mg) and succinic anhydride (226 mg) were dissolved in 40 mL anhydrous CH_2Cl_2. DMAP (144 mg) dissolved in 10 mL CH_2Cl_2 was added dropwise into the solution and the reaction was stirred at 45 ℃ for 6 h. Then the mixture was washed with saturated sodium bicarbonate solution. The crude product was purified with silica gel column chromatography to obtain VES-SS-COOH. The chemical structure was confirmed by ^{1}H NMR (400 MHz, $CDCl_3$).

1.1.5 Synthesis of PEI-SS-VES

To obtain PEI-SS-VES, COOH-SS-VES (63.6 mg), EDC (222.4 mg), and NHS (133.4 mg) were dissolved in 20 mL of anhydrous DMSO and stirred for 30 min. Then, PEI (100 mg) dissolved in 5 mL DMSO was added dropwise to the solution, and the reaction was stirred for 24 h. The reaction was dialyzed in deionized water for 72 h. The dialysate was then lyophilized to obtain the final product. The chemical structure was confirmed using ^{1}H NMR (400 MHz, DMSO-d6). We also synthesized PEI-VES without a disulfide bond according to our previous study [1].

1.2. Synthesis of HA-PEG-RGD

1.2.1 Synthesis of thiolated HA (HA-SH)

HA (200 mg) was dissolved in 10 mL deionized water over night. Then EDC (19 mg) and cysteamine (3.9 mg) were added into above solution. The pH was adjusted to 5.3 by the addition of 1 M NaOH and the reaction was stirred for 2 h. The same amount of EDC and cysteamine were added into the solution and stirred for another 2 h. Then the reaction was dialyzed (molecular weight cut-off: 3.5 kDa) against deionized water containing 0.25 mM TCEP for 72 h. The product was obtained after

lyophilization. The chemical structure was confirmed by ^{1}H NMR (400 MHz, D_2O).

1.2.2 Synthesis of HA-PEG-RGD

Hyaluronic acid was thiolated to obtain HA-SH, as described in the Supplementary Information. Then, MAL-PEG_{2000}-NHS (50 mg) and HA-SH (200 mg) were dissolved in 10 mL of deionized water. The pH was adjusted to 7 and the reaction was stirred for 12 h. Then, RGD (15 mg) was added to the solution, the pH was adjusted to 8, and the reaction was stirred for an additional 12 h. The reaction was dialyzed in deionized water for 72 h. The product was obtained following lyophilization. The chemical structure was confirmed using ^{1}H NMR (400 MHz, D_2O). We also synthesized HA-PEG without RGD moiety according to our previous study [1].

1.3 DNase protection assay of pDNA@PVHRs NP

The 16 μL of pDNA@PVHRs NP were incubated with 2 μL of DNase I solution (1 U/μL) and 2 μL of 10 × DNase reaction buffer (100 mM Tris-HCl, 1 mM $CaCl_2$ and 25 mM $MgCl_2$) for different time (0 h, 1 h, 3 h, 6 h, 12 h and 24 h) at 37 ℃ [2]. Then 3 μL of EDTA (0.5 M) was added to inactivate DNase I for 10 min at room temperature. The samples were treated with heparin (10 mg/mL) for 1 h at 37 ℃ to dissociate pDNA from nanoparticles. Finally, the resulting samples were detected by a 0.6% agarose gel electrophoresis assay.

1.4 Hemolysis assay of PVHRs NP

The hemocompatibility of PVHRs NP was investigated by hemolysis assay. 1 mL blood samples obtained from Balb/c mice by heart puncture were diluted with 2 mL PBS. Red blood cells were collected by centrifuging at 3000 rpm for 15 min at 4°C. After being washed for 3 times, 40 μL of

red blood cells suspension was mixed with 960 μL of PBS, deionized water and PVHRs NP with a concentration range from 0.05 to 0.5 mg/mL. After incubating at 37 ℃ for 30 min, the mixtures were centrifuged at 5000 rpm for 5 min. 100 μL of the supernatant was monitored by a microplate reader at 540 nm. The erythrocytic suspensions diluted with saline and 2% Triton X-100 were defined as negative control and positive control, respectively.

1.5 Pharmacokinetics

Balb/c mice were randomly divided into 5 groups (n=3 per group) and then treated with DIR, DIR@PVs micelles, DIR@PVHs NP, DIR@PVHPs NP, or PVHRs NP, at a dose of 100 μg/kg. Blood samples (100 μL in volume) were subsequently collected from the eye sockets at 1, 5, 15, 30, 60, 120, 240, 480, and 1440 min post-treatment. Plasma was collected by centrifuging the blood samples at 6,000 rpm for 10 min. DIR concentration in each sample was measured by a microplate reader (Synergy 2, BioTek, USA) set to 740/790 nm excitation/emission. The values of the concentrations of DIR in individual animals were then uploaded into Kinetica 5.1 software for the calculation of a range of pharmacokinetic parameters. The software was used to fit the data to a two-compartment model, and the proposed model was then used to determine the pharmacokinetic parameters.

1.6 Biodistribution analysis

Mice bearing 4T1 tumors were injected intravenously with DIR-labeled PVs micelles, PVHs NP, PVHPs NP, or PVHRs NP, at a dose of 100 μg/kg. Mice were subsequently sacrificed at different time points (4 h, 8 h and 24 h). Following sacrifice, the major organs and tumors were excised,

rinsed in saline, weighed, and homogenized. The concentration of DIR in individual tissues or tumors was then determined using a microplate reader. For *ex vivo* imaging, mice were sacrificed at 24 h post-injection; major organs and tumors were excised and imaged using the In-Vivo Multispectral System FX.

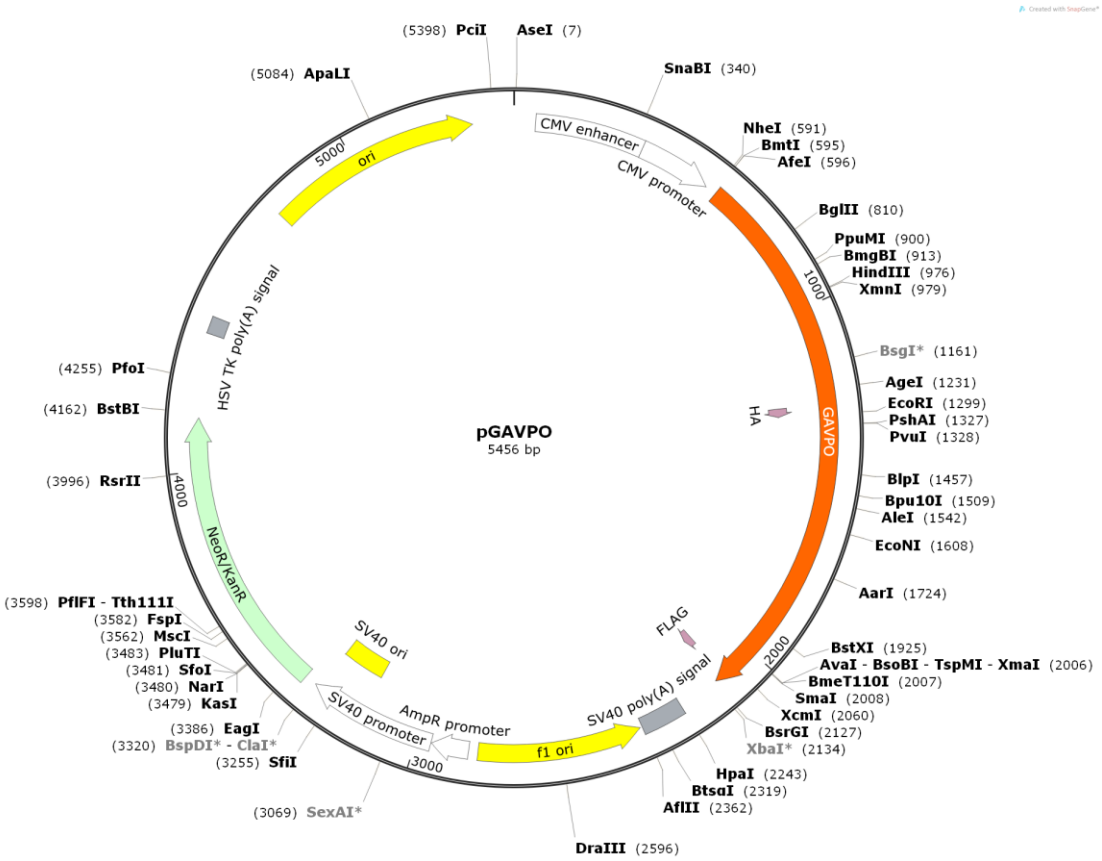

Figure S1. Sequence map of pGAVPO.

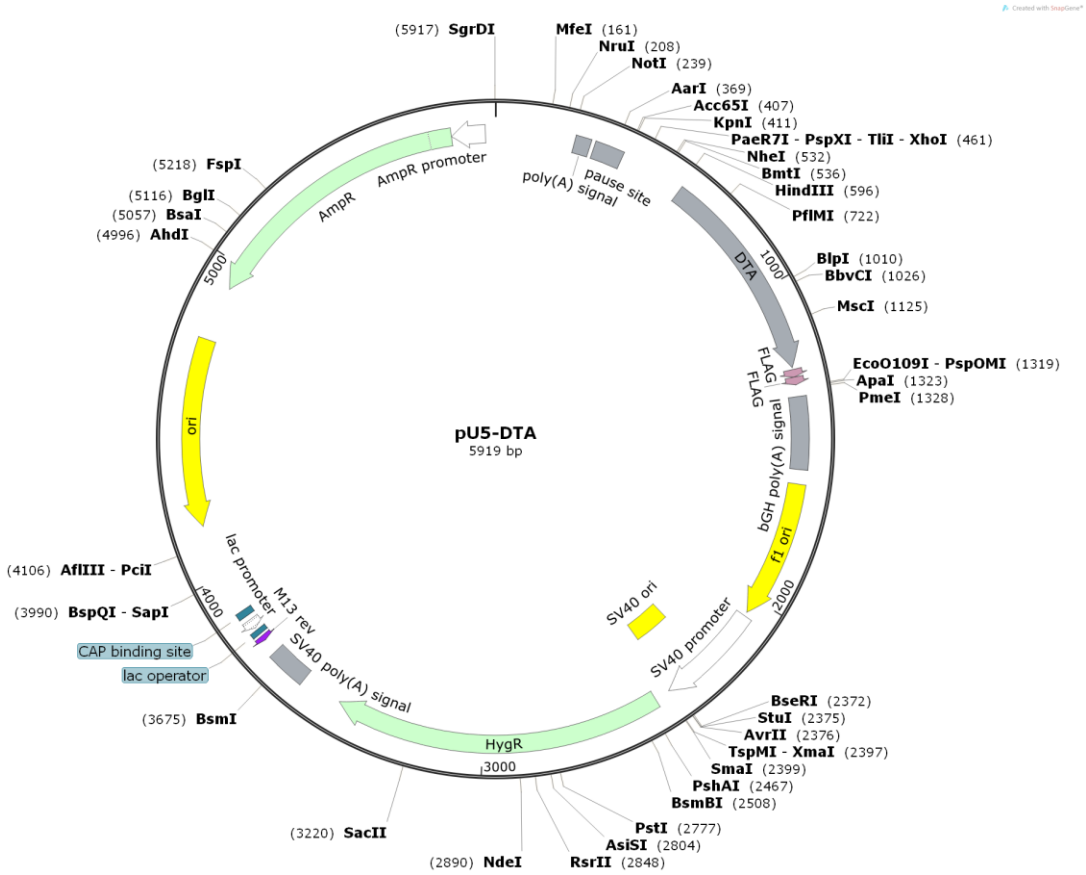

Figure S2. Sequence map of pU5-DTA.

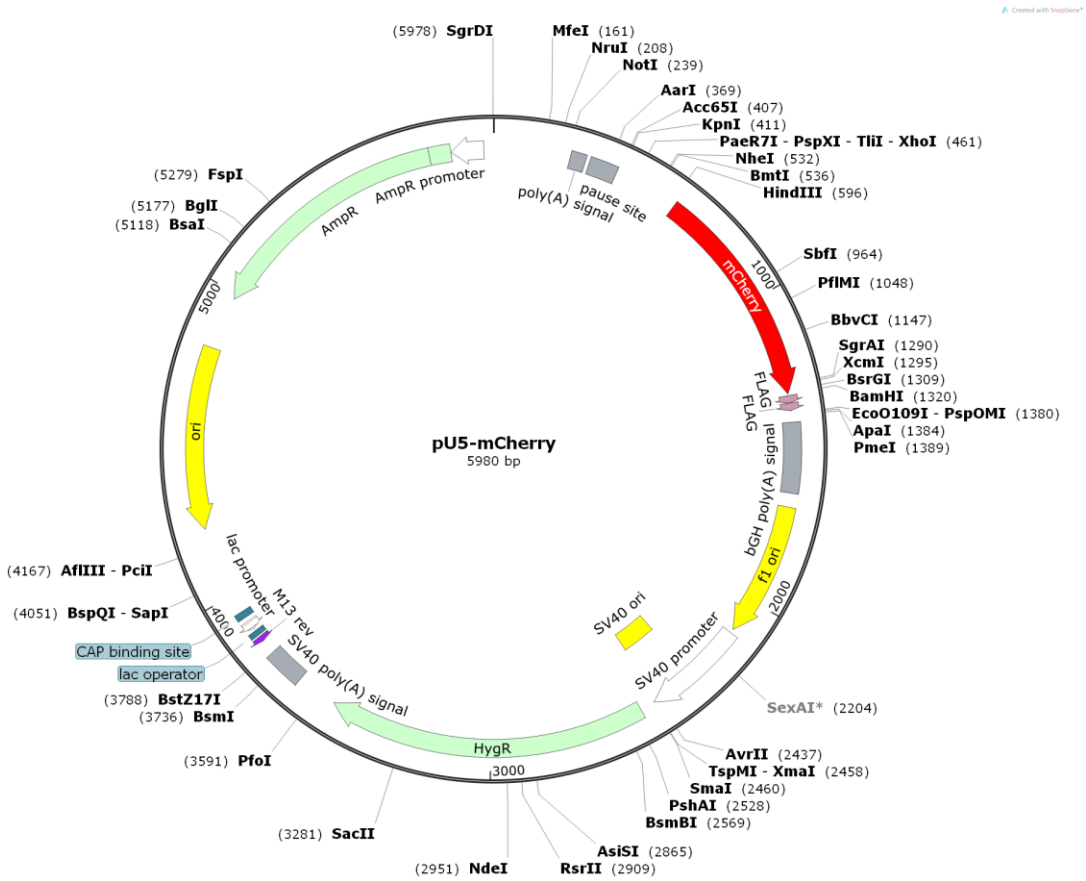

Figure S3. Sequence map of pU5-mCherry.

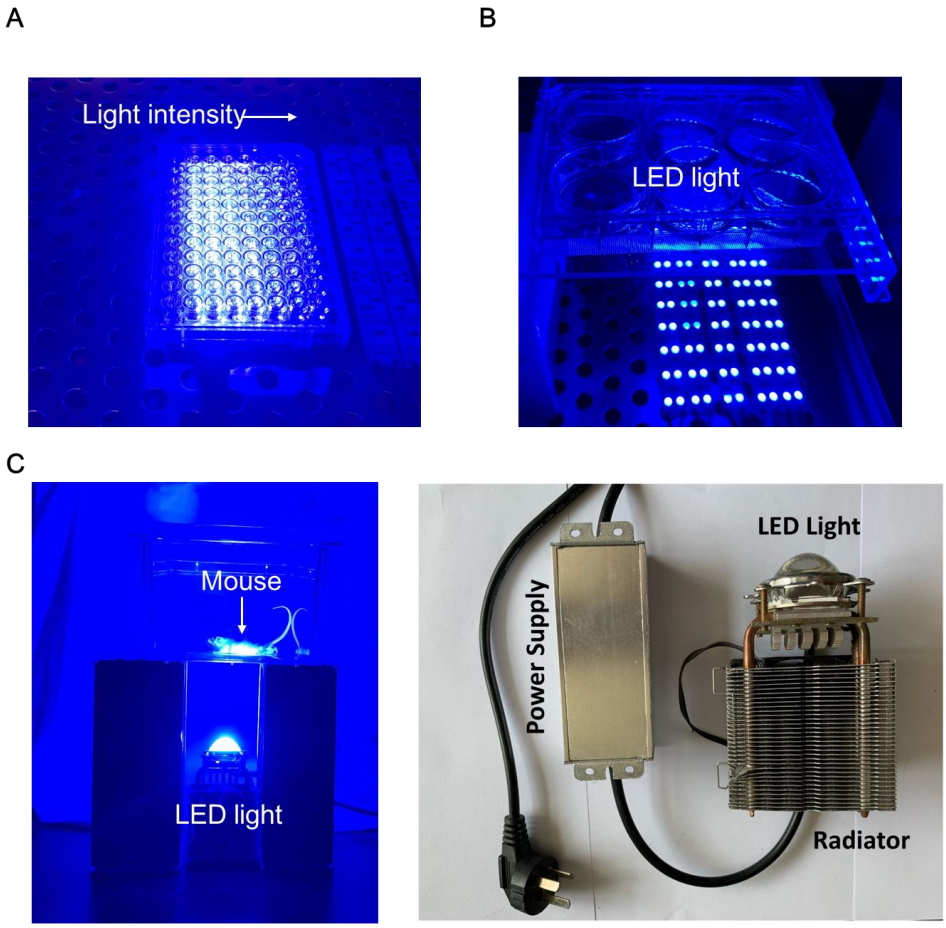

Figure S4. (A) Intensity controlled LED arrays for cell cultures. (B) Timer controlled LED arrays for cell cultures. (C) Timer controlled LED arrays for *in vivo* irradiation and laser devices used for light-switchable transgene expression (90 W/m^2).

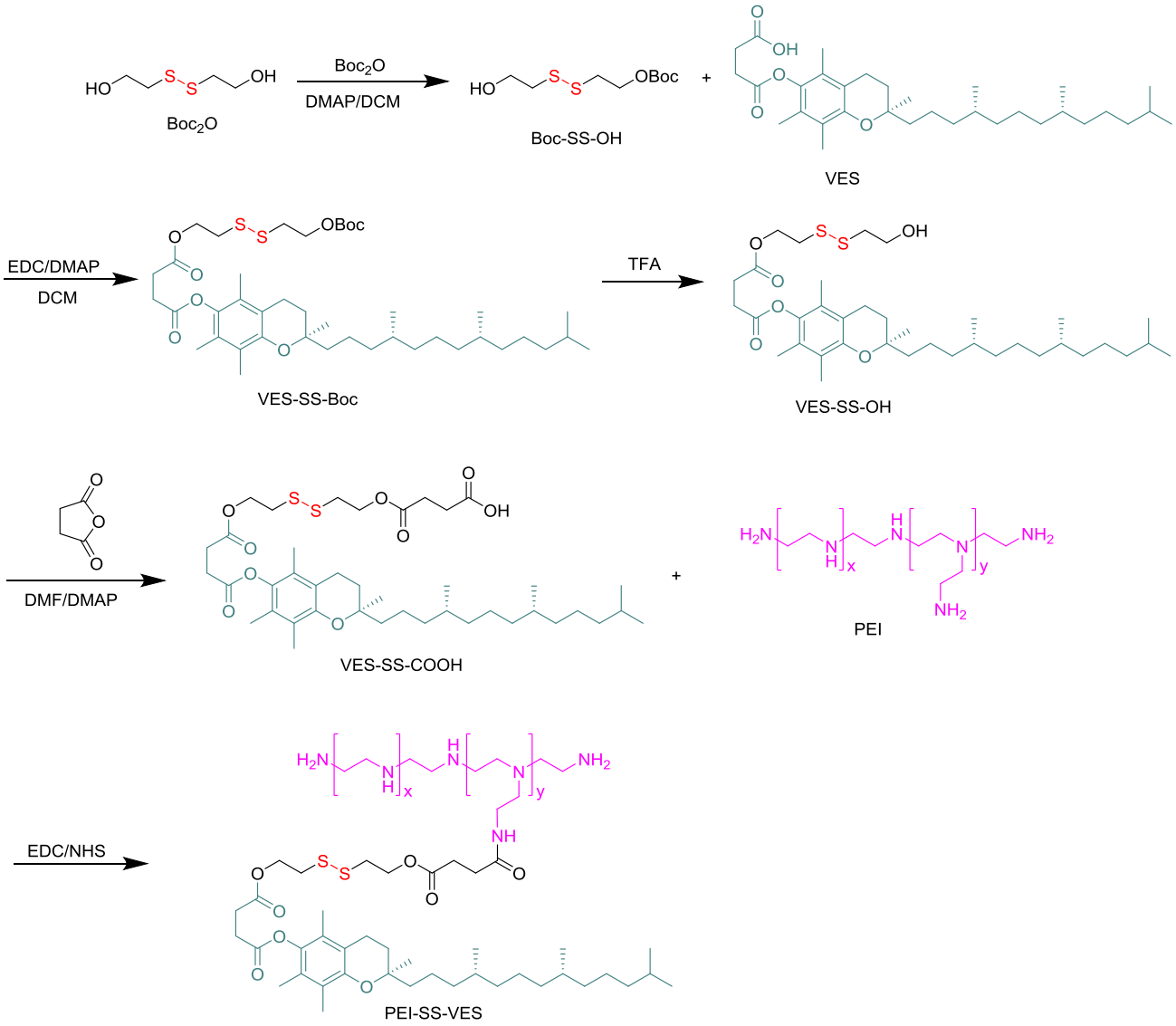

Figure S5. Synthesis route of PEI-SS-VES.

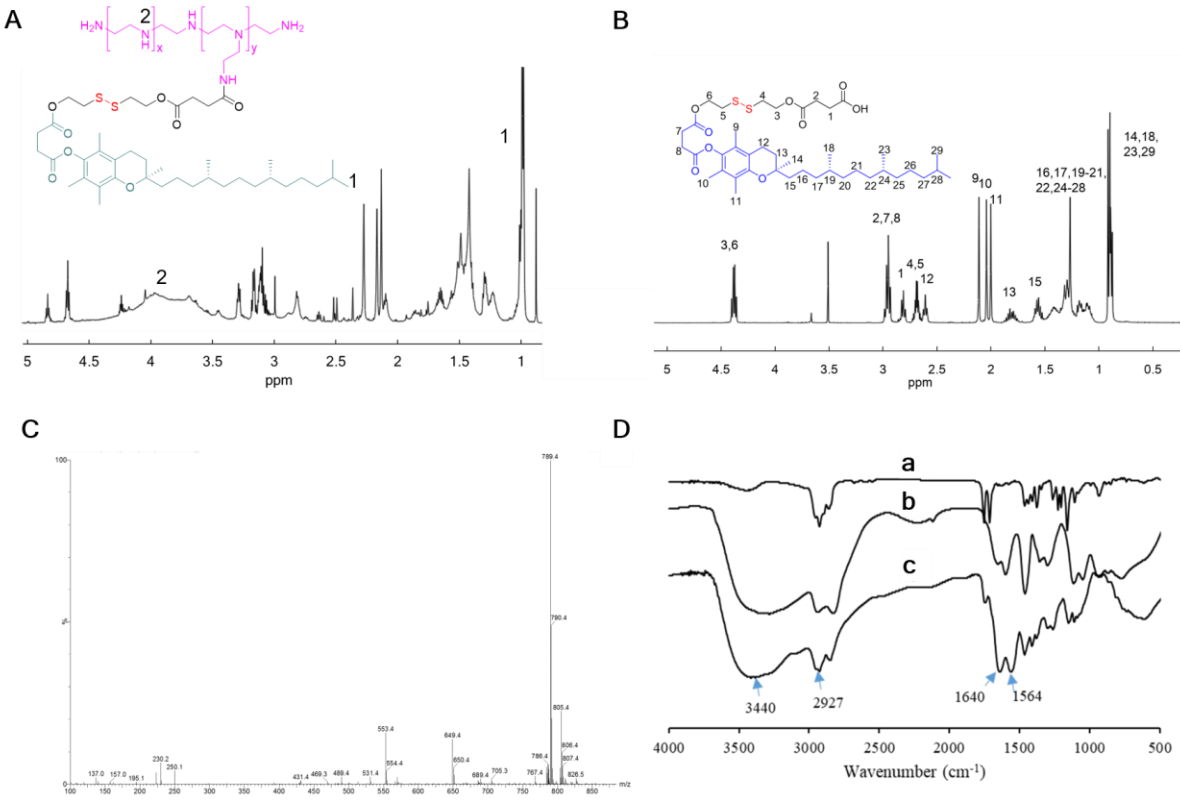

Figure S6. (A) ^{1}H NMR of PEI-SS-VES. (B) ^{1}H NMR and (C) ESI-mass spectra of VES-SS-COOH. (D) Infrared spectra of (a) VES, (b) PEI and (c) PEI-SS-VES.

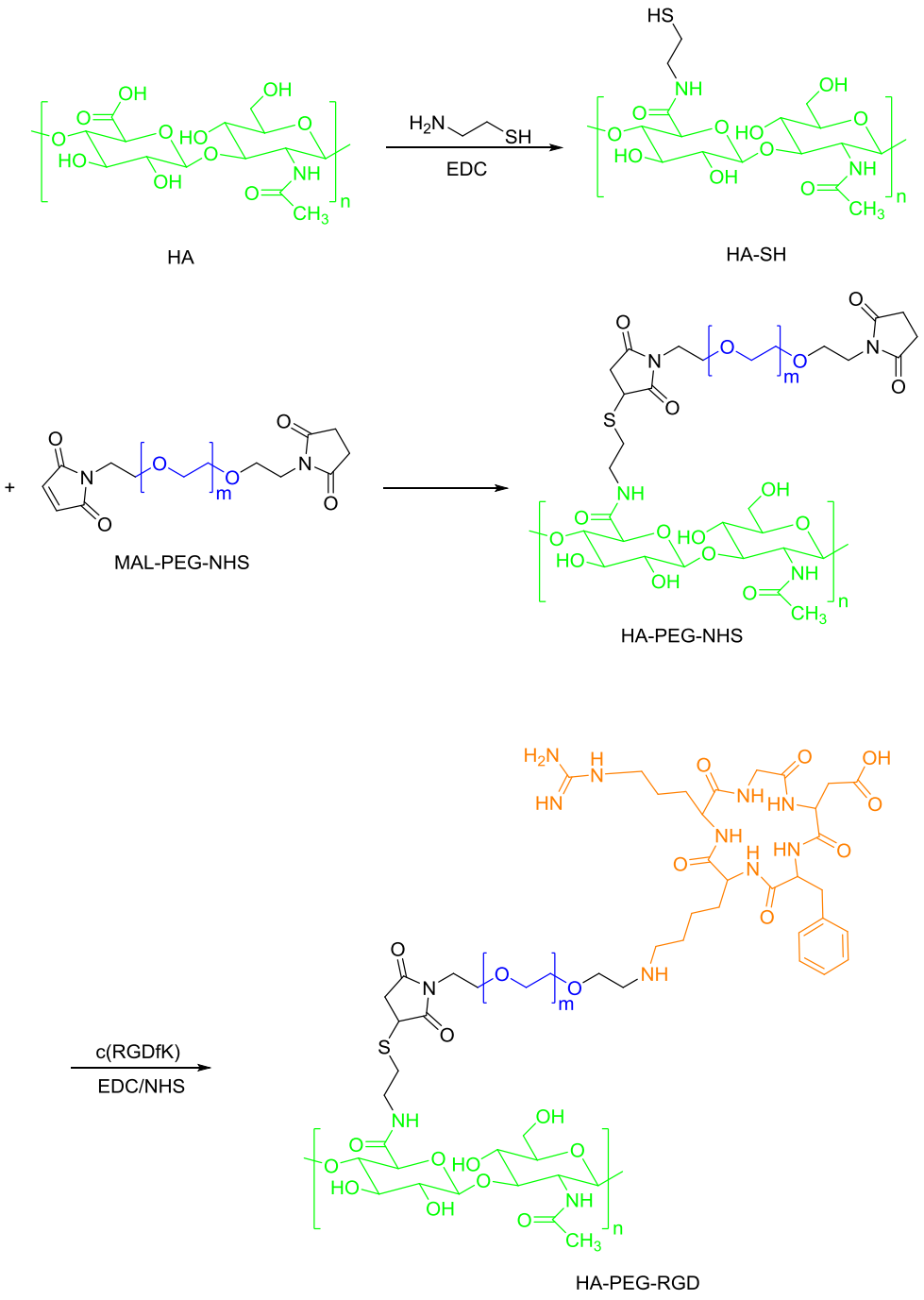

Figure S7. Synthesis route of HA-PEG-RGD.

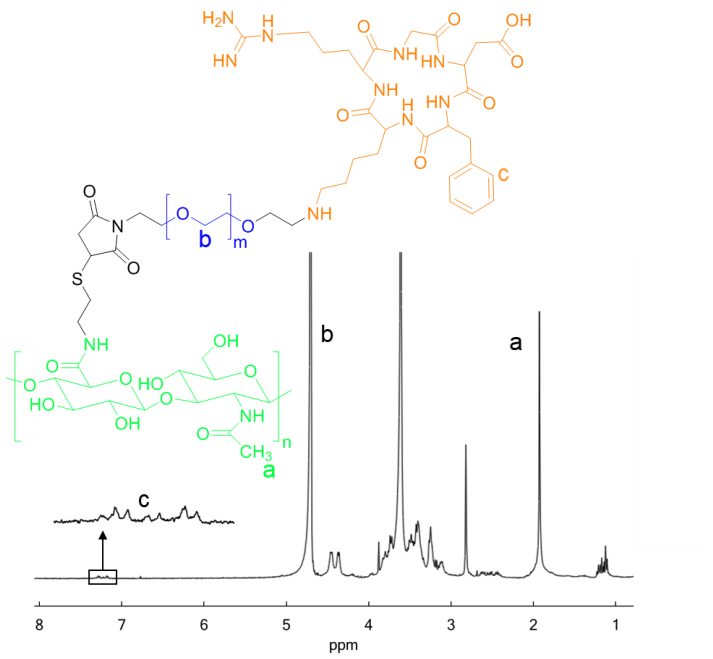

Figure S8. ^{1}H NMR of HA-PEG-RGD.

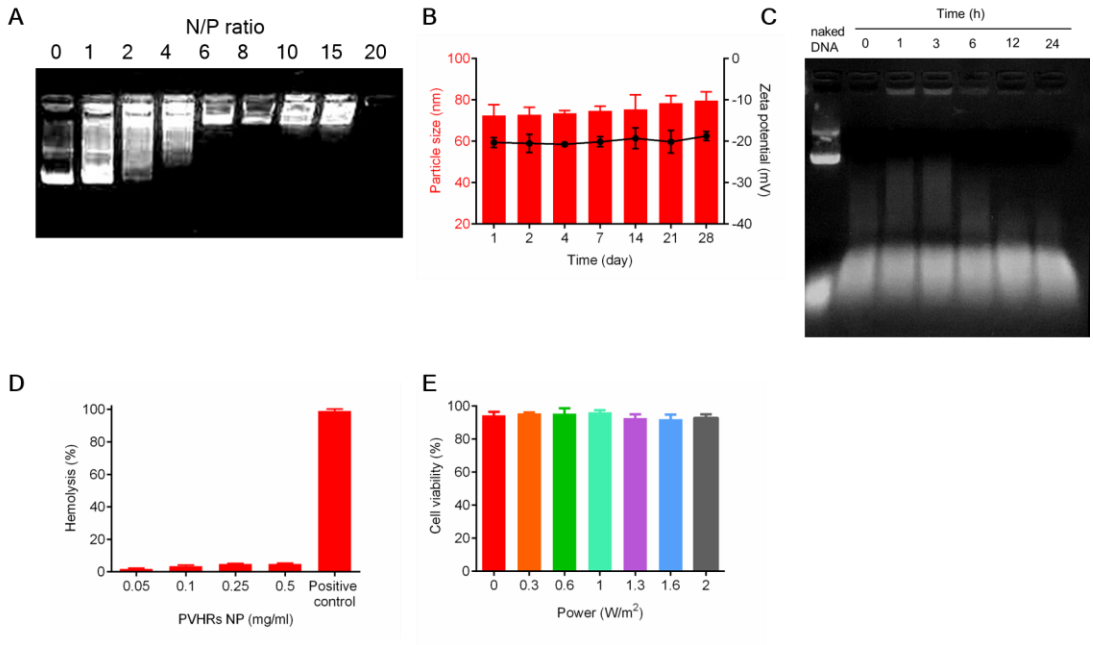

Figure S9. (A) Gel electrophoresis assay of pGDTA@PVs micelles at different N/P ratio. (B) Particle size and zeta potential changes of pDNA@PVHRs NP at pH 5.5. Data were presented as mean ± SD (n = 3). (C) Protection assay of pGDTA@PVHRs NP against DNase I for different time. (D) Hemolysis assay of PVHRs NP with different concentration, 2% Triton X-100 was used as positive control. Data were presented as mean ± SD (n = 3). (E) Cell viability of 4T1 cells after 48 h incubation with PVHRs NP under blue light radiation. Data were presented as mean ± SD (n = 3).

Table S1 Physicochemical properties of PVHRs NP prepared at different feeding weight ratios of PEI-SS-VES relative to HA-PEG-RGD.

PEI-SS-VES/HA-PEG-RGD (w/w)	Particle size (nm)	Zeta potential (mV)	PI
1/0	65.2 ± 1.3	32.5 ± 2.5	0.10
1/1	68.5 ± 0.2	-10.7 ± 0.7	0.21
1/2	76.7 ± 1.3	-15.5 ± 0.4	0.20
1/4	74.3 ± 1.2	-20.3 ± 3.1	0.14
1/8	75.8 ± 5.7	-20.5 ± 2.1	0.15

PI: polydispersity index. Data were presented as mean ± SD (n = 3).

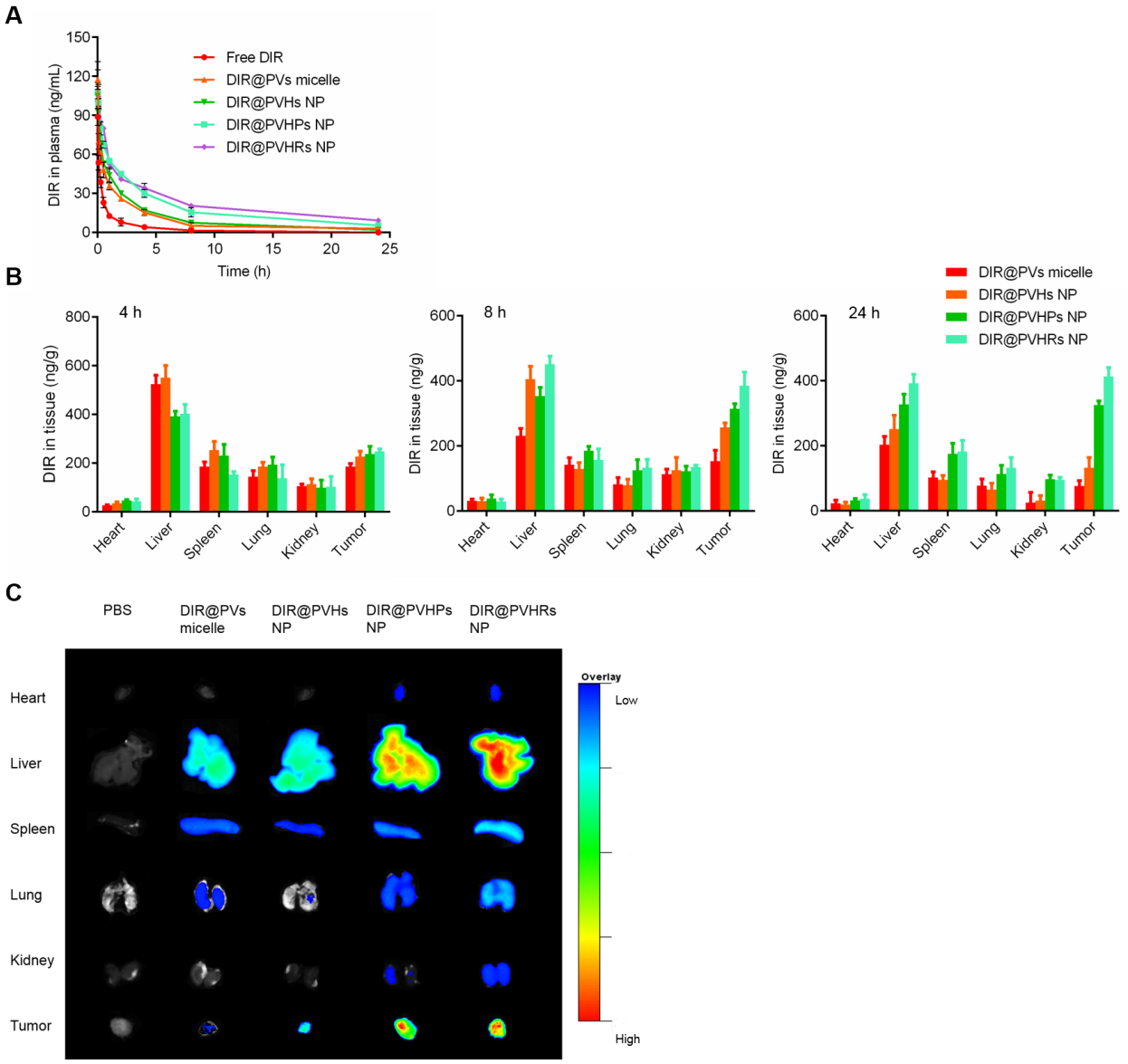

Figure S10. (A) Concentration-time profile in plasma after intravenous injection of DIR, DIR@PVs micelle, DIR@PVHs NP, DIR@PVHPs NP or DIR@PVHRs NP in Balb/c mice at the dose of 100 μg/kg. Data were presented as mean ± SD (n = 3). (B) Biodistribution of DIR in 4T1 bearing Balb/c mice at 4 h, 8 h and 24 h after intravenous injection with different DIR loaded nanoparticles at the dose of 100 μg/kg. Data were presented as mean ± SD (n = 3). (C) *Ex vivo* fluorescence images of heart, liver, spleen, lung, kidney and tumor from 4T1 bearing Balb/c mice at 24 h after intravenous injection with different DIR loaded nanoparticles.

Table S2 Pharmacokinetic parameters in plasma of DIR after intravenous injection of DIR, DIR@PVs micelles, DIR@PVHs NP, DIR@PVHPs NP or DIR@PVHRs NP in Balb/c mice at the dose of 100 μg/kg.

Pharmacokinetic parameters	DIR	DIR@PVs micelles	DIR@PVHs NP	DIR@PVHPs NP	DIR@PVHRs NP
$t_{1/2\alpha}$ (h)	0.2 ± 0.0	0.4 ± 0.2	0.9 ± 0.1	1.0 ± 0.3	0.5 ± 0.2
$t_{1/2\beta}$ (h)	2.1 ± 0.2	7.1 ± 1.5	8.1 ± 0.8	9.9 ± 1.4	11.7 ± 2.1
$AUC_{0\rightarrow 24h}$ ($\mu g \cdot h \cdot L^{-1}$)	66.1 ± 3.5	226.5 ± 32.1*	256.0 ± 22.3*	437.5 ± 38.7**	529.9 ± 47.5**
MRT (h)	2.5 ± 0.2	7.8 ± 1.6*	7.7 ± 0.3*	10.9 ± 1.5**	14.5 ± 2.3**
CL (L h^{-1})	1.5 ± 0.3	0.4 ± 0.2	0.36 ± 0.11	0.2 ± 0.1	0.2 ± 0.1

$t_{1/2\alpha}$: distribution half-life time; $t_{1/2\beta}$: elimination half-life time; AUC: area under the curve; MRT: mean residence time; CL: clearance. *$p < 0.05$, **$p < 0.01$ versus DIR treatment. Data were presented as mean ± SD (n = 3).

Table S3 $AUC_{0\text{-}24h}$ of DIR in main organs and tumor after intravenous injection of DIR@PVs micelles, DIR@PVHs NP, DIR@PVHPs NP or DIR@PVHRs NP in Balb/c mice at the dose of 100 μg/kg.

Formulation	$AUC_{0\text{-}24h}$ ($\mu g\ h\ g^{-1}$)					
	Heart	Liver	Spleen	Lung	Kidney	Tumor
DIR@PVs micelles	0.6 ± 0.1	6.0 ± 0.6	3.0 ± 0.4	2.0 ± 0.5	1.8 ± 0.4	2.9 ± 0.5
DIR@PVHs NP	0.6 ± 0.2	8.3 ± 0.9	3.1 ± 0.4	2.1 ± 0.4	2.0 ± 0.6	4.5 ± 0.5*
DIR@PVHPs NP	0.8 ± 0.2	7.7 ± 0.6	4.2 ± 0.6	2.9 ± 0.7	2.4 ± 0.4	6.5 ± 0.4**
DIR@PVHRs NP	0.8 ± 0.2	9.3 ± 0.6	3.6 ± 0.7	2.9 ± 0.7	2.5 ± 0.3	8.1 ± 0.7**

AUC: area under the curve. *$p < 0.05$, **$p < 0.01$ versus DIR@PVs micelle. Data were presented as mean ± SD (n = 3).

Reference

[1] He MY, Huang L, Hou XY, Zhong C, Bachir ZA, Lan MB, et al. Efficient ovalbumin delivery using a novel multifunctional micellar platform for targeted melanoma immunotherapy. *Int J Pharm* 2019;**560**:1-10.

[2] Chen KR, Cao XQ, Li M, Su YJ, Li HP, Xie MY, et al. A tRAIL-delivered lipoprotein-bioinspired nanovector engineering stem cell-based platform for inhibition of lung metastasis of melanoma. *Theranostics* 2019;**9**:2984-98.