Supporting Information

Host-cell-assisted construction of folate-engineered nanocarrier based on viral light particle for targeted cancer therapy

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1. Experimental Section

1.1. Characterization of Component of F-L-particles

Purified folate-engineered L-particles (F-L-particles) were fixed on pre-rinsed glass coverslips (22×22 nm) by heating. After washing with ultrapure water, F-L-particles were incubated with 100 μL of the primary antibody against pseudorabies virus (PrV) at 37°C for 1 h. To remove excess antibody, the samples were washed thrice with 1×PBS extensively at room temperature. The samples were incubated with DyLight 649 conjugated secondary antibody at 37°C for 40 min and then washed thrice with 1×PBS. Subsequently, viral envelope was labeled with 100 μL of 5 μM DiO and then 5 ng/mL SYTO 82 at room temperature for 40 min and washed with 1×PBS for use. The prepared samples were observed by a spinning-disk confocal microscope (Andor Revolution XD).

1.2. Cell Viability and Proliferation Assays

HeLa cells seeded in 96- and 12-well plates were incubated with 1×PBS (control), QDs, L-particles and QDs@F-L-particles (protein content, 20 μg/mL) for different hours for cell viability and proliferation assays, respectively. The cell viability was measured by MTT assay. Digested cells were counted by hemocytometer.

1.3. Whole Blood Analysis and Histological Examination

Age- and weight-matched female Balb/c mice were injected with 100 μL of 1×PBS (control), L-particles, QDs, and QDs@F-L-particles (protein content, 1 mg/mL, n=5) *via* tail vein, respectively. The injection was repeated every 3 days for up to 5 times. The body weights of mice were recorded every 3 days. After 3 weeks, the mice were euthanized. 200 μL of blood samples from each mouse were collected for further whole blood analysis. The organs harvested from each mouse were fixed in 4% paraformaldehyde for 24 h, and then embedded, sliced and stained with H&E. The samples were observed with a CCD camera (Nikon digital sight DS-U3) mounted on an inverted fluorescence microscope (Ti, Nikon).

2. Supplementary Figures

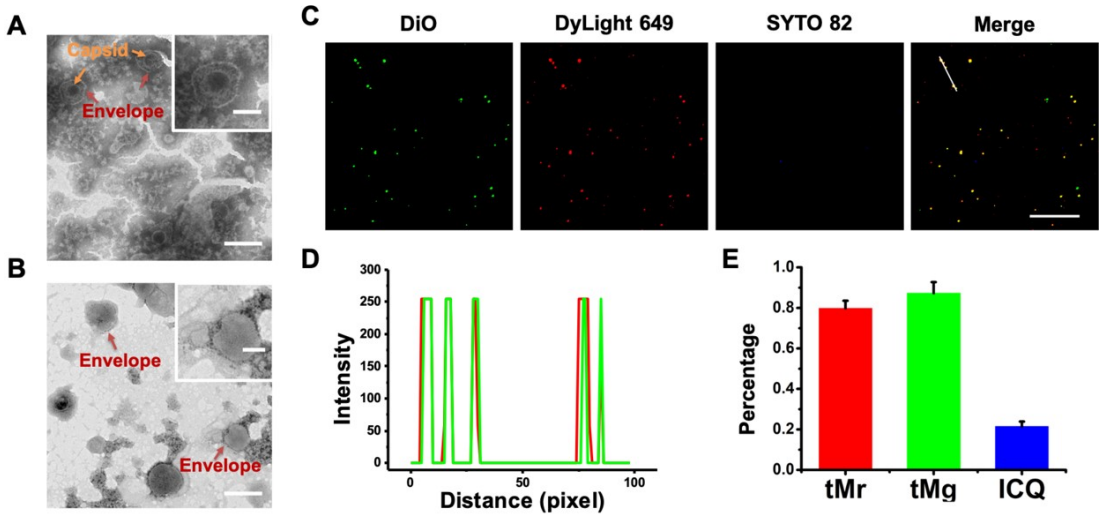

Fig. S1 (A, B) TEM images of mature PrV virions (A) and viral L-particles (B). Scale bars: 250 nm. Inset: enlarged TEM images of a single mature PrV virion (A) and viral L-particle (B). Scale bars: 100 nm. (C) Confocal images of DiO labeled envelopes (green), DyLight 649 immuno-labeled PrV envelope proteins (red), SYTO 82 labeled DNA (blue), and their merged image on glass coverslips. Scale bar: 20 μm. (D) Line profile of the distribution and intensity of DiO (green) and DyLight 649 (red) signals on the line in C. (E) Histograms of Manders coefficient for red (tMr) and green (tMg) signals in thresholded image and the intensity correlation quotient (ICQ) corresponding to the signals of Dylight 649 and DiO (n=10).

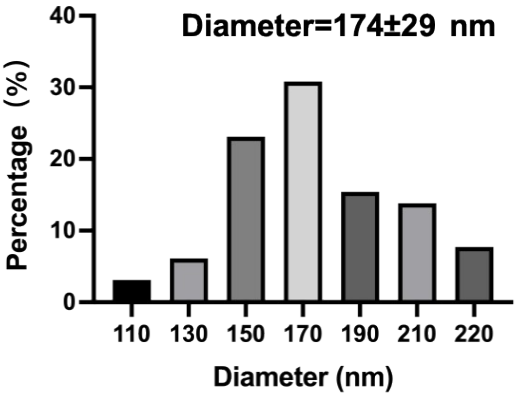

Fig. S2 Statistical analysis of the diameter of F-L-particles according to TEM images (n=65).

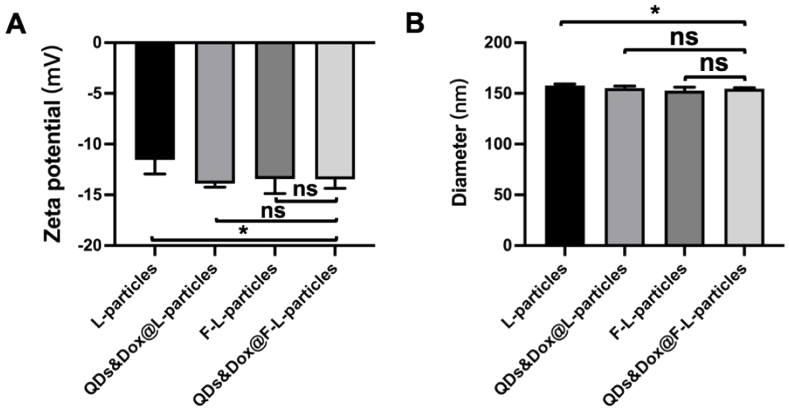

Fig. S3 (A) Zeta potential of L-particles, QDs&Dox@L-particles, F-L-particles, and QDs&Dox@F-L-particles (n=5). (B) Average diameter of L-particles, QDs&Dox@L-particles, F-L-particles, and QDs&Dox@F-L-particles calculated by dynamic light scattering (DLS) (n=3). **P*<0.05. ns indicates no significant difference.

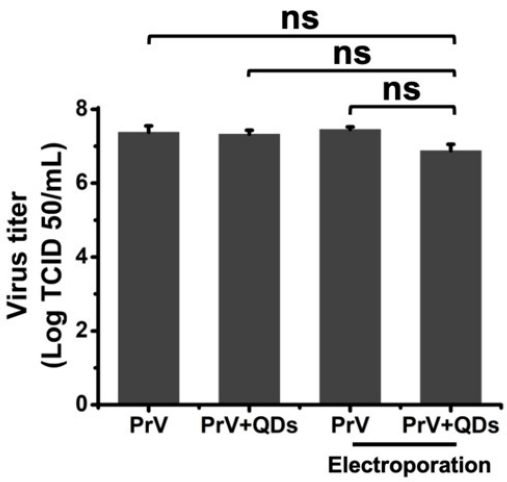

Fig. S4 Infectivity of PrV incubated with Ag_2Se QDs without/with electroporation. L-particles have no genome, making it impossible to evaluate effect of electroporation on the infectivity of L-particles directly, therefore, PrV was selected as an alternative. The virus titers in all conditions were kept in the same order of magnitude results indicating that neither incubation with QDs nor electroporation had side effect on the infectivity of PrV. ns indicates no significant difference.

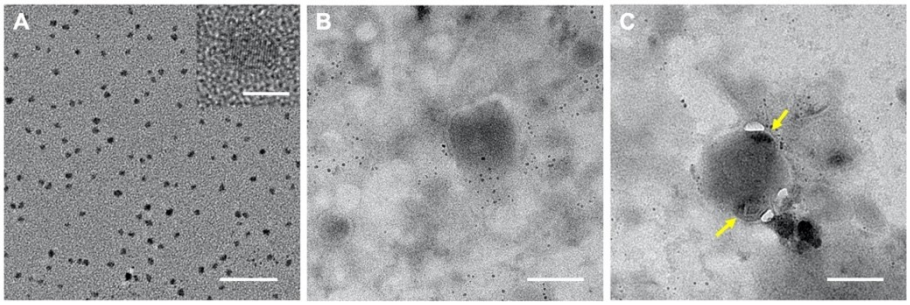

Fig. S5 (A) HRTEM image of Ag_2Se QDs. Scale bar: 50 nm. Inset: HRTEM image of a single Ag_2Se QD. Scale bar: 5 nm. (B and C) HRTEM images of F-L-particles incubated Ag_2Se QDs without and with electroporation. Scale bars: 100 nm.

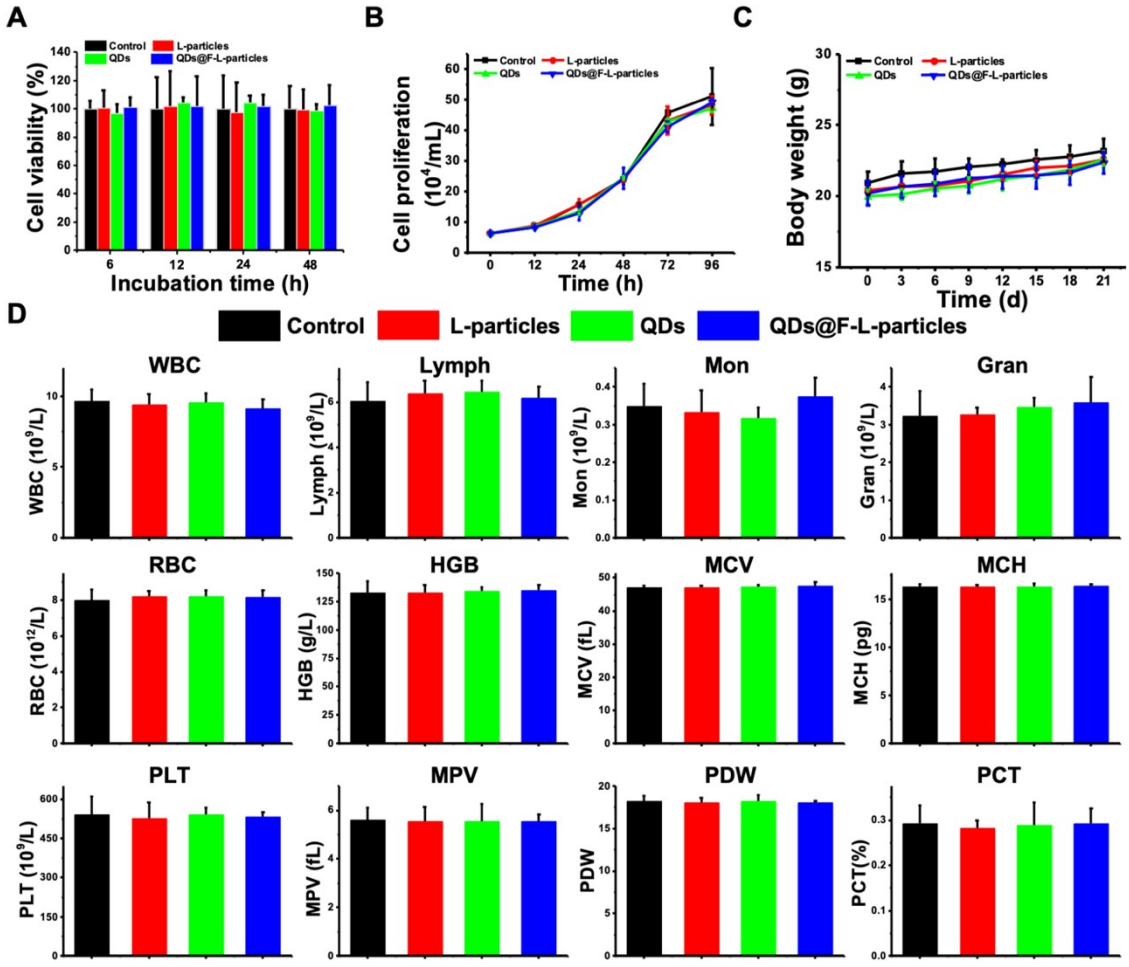

Fig. S6 (A) Cell viability of HeLa cells incubated with DMEM containing 10% FBS (control), L-particles, QDs, and QDs@F-L-particles for different hours (n=9). Relevant *P* values have been calculated by T-test, and are all above 0.05, indicating no significant difference in all cases. (B) Proliferation of HeLa cells incubated with DMEM containing 10% FBS (control), L-particles, QDs, and QDs@F-L-particles for different hours (n=3). (C) Body weights of mice after intravenously injecting 1×PBS (control), L-particles,

QDs, and QDs@F-L-particles (n=5). (D) Whole blood cell analysis of mice after being intravenously injected with 1×PBS (control), L-particles, QDs, and QDs@F-L-particles for 3 weeks (n=5). WBC, white blood cell; Lymph, lymphocyte; Mon, monocyte; Gran, granulocyte; RBC, red blood cell; HGB, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; PLT, platelets; MPV, mean platelet volume; PDW, platelet distribution width; PCT, plateletcrit.

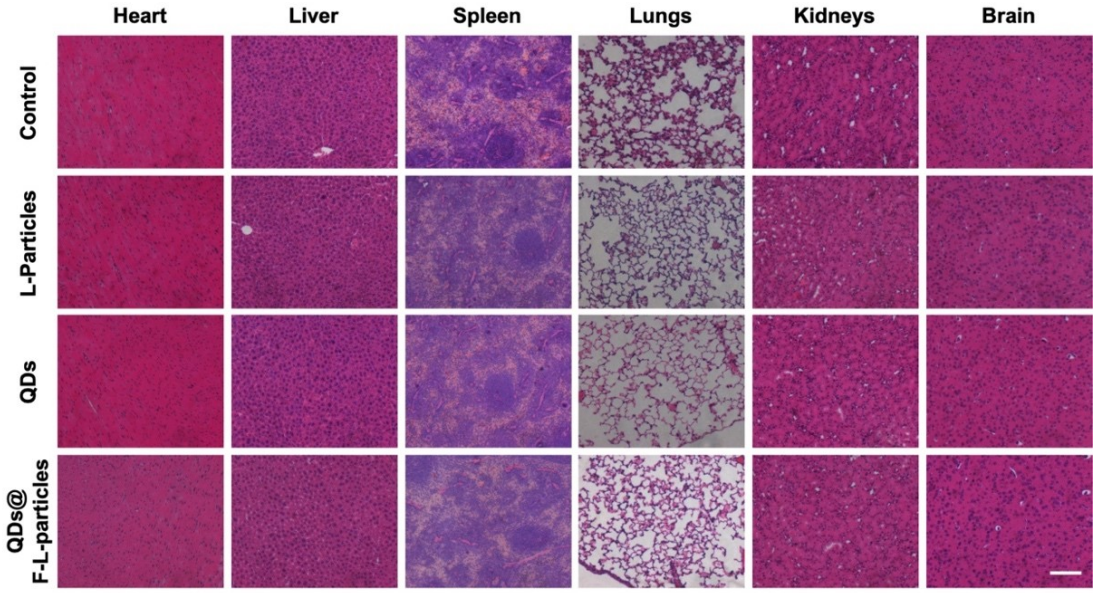

Fig. S7 H&E staining of major organs, including heart, liver, spleen, lung, kidney and brain, dissected from mice intravenously injected with 1×PBS (control), L-particles, QDs and QDs@F-L-particles for 3 weeks, respectively. Scale bar: 100 μm.

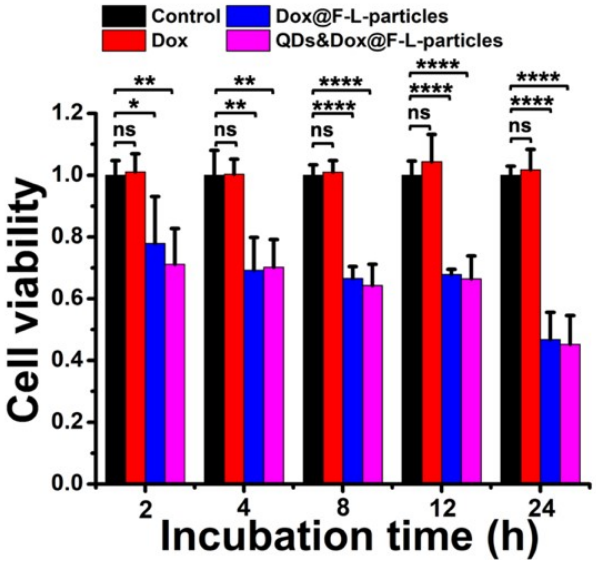

Fig. S8 Cell viability of HeLa cells incubated with Dox, Dox@F-L-particles, and Dox&QDs@F-L-particles for different hours (n=9). The cell viability of the control was normalized to 100%. ns indicates there is no significant difference. *P<0.05. **P<0.01. ****P<0.001.

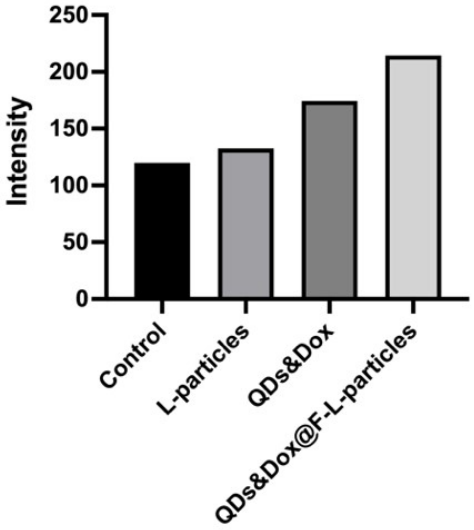

Fig. S9 Fluorescence intensity of the tumor sites in different groups corresponding to Fig. 5B.

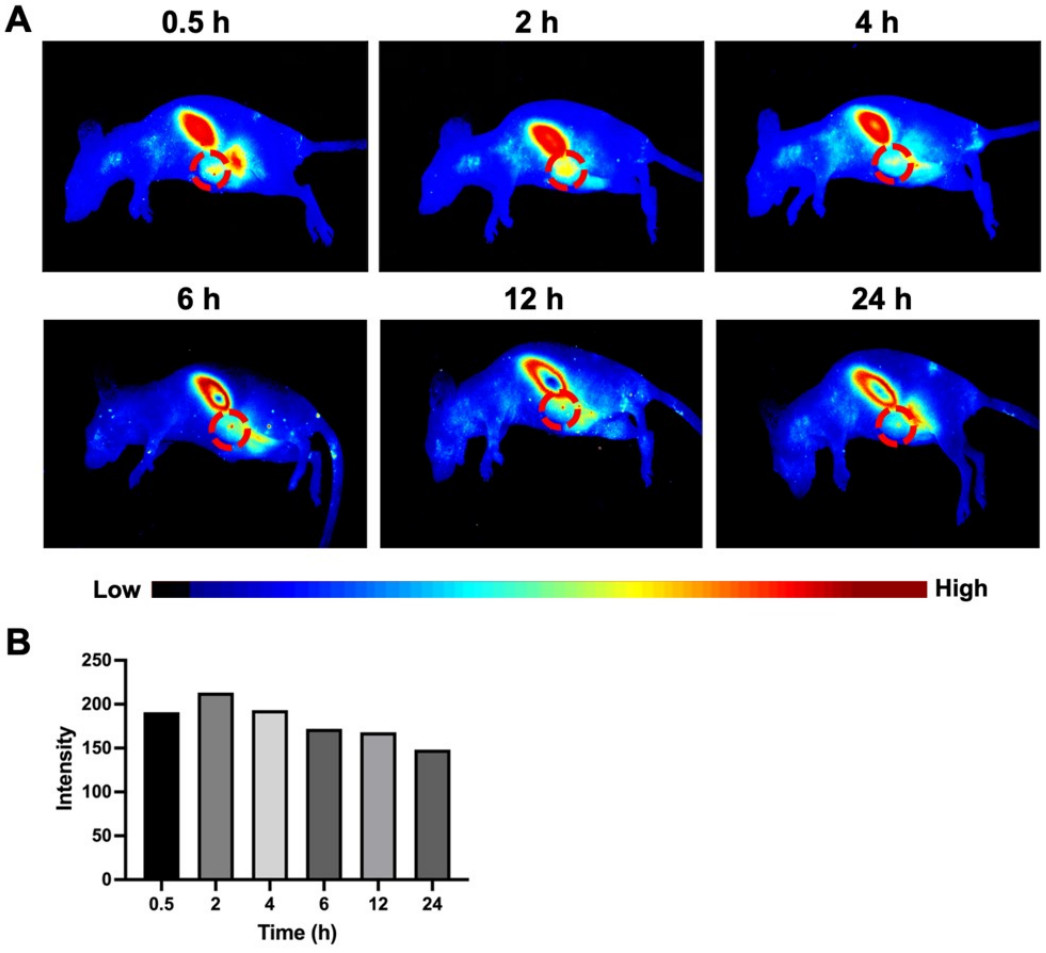

Fig. S10 (A) Near-infrared fluorescence images of tumor-bearing nude mice intravenously injected with QDs&Dox@F-L-particles for different hours. The red circles indicate the tumor sites. (B) Fluorescence intensity of the tumor sites at different times corresponding to A.