

Supporting Information

Sequential Intra-Intercellular Delivery of Nanomedicine for Deep Drug-Resistant Solid Tumor Penetration

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Experimental Section

Immunohistochemical assay. For CD31 and Ki67 immunostaining, antigen retrieval was performed using discovery CC1 solution (Ventana) at 95°C (pH 8.5). The primary antibody of Ki67 (MAB-0672, Maixin. Bio, Fujian, China) was incubated for 32 min at 37 °C with a dilution of 1:100. Following staining, sections were observed using a microscope to obtain the proliferation index (percentage of Ki67-positive cells from the total cells). Three fields with the highest density of immunolabeled tumor cell nuclei (“hot-spot”) were assessed per tumor at 10× magnification. The procedure for CD31 staining was similar to that for Ki67 staining. The anti-CD31 primary antibody (MAB-0672, Maixin. Bio, Fujian, China) was used.

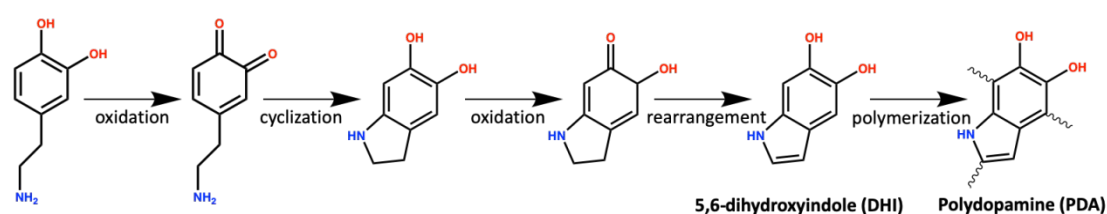


Figure S1. Polymeric mechanism of DA to PDA (DHI is treated as the primary molecular in the polymerization).

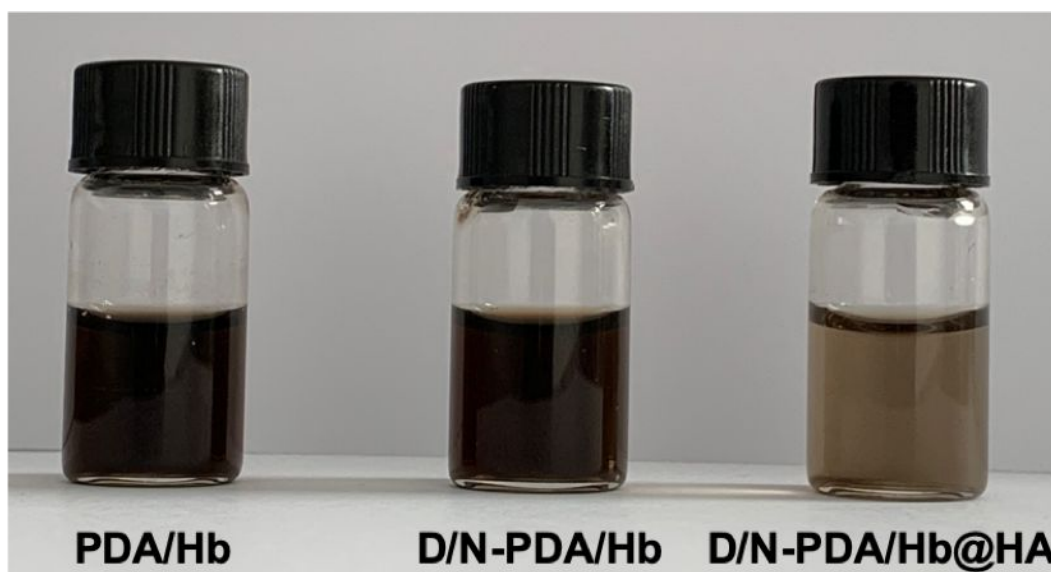


Figure S2. Photographs of PDA/Hb, D/N-PDA/Hb and D/N-PDA/Hb@HA

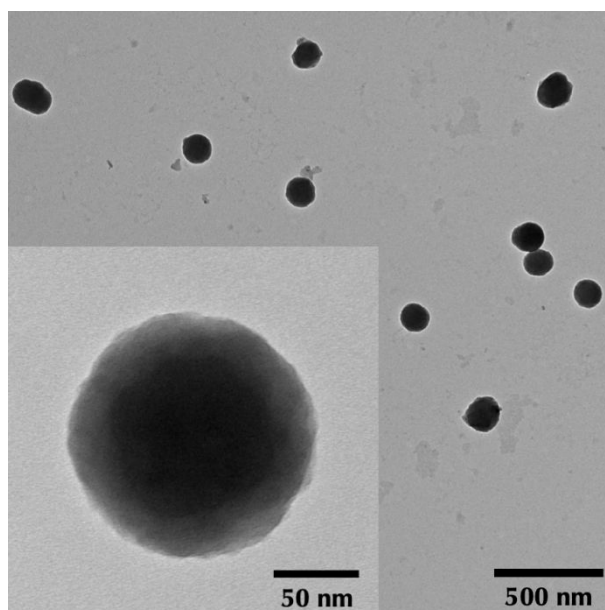


Figure S3. TEM images of D/N-PDA/Hb@HA.

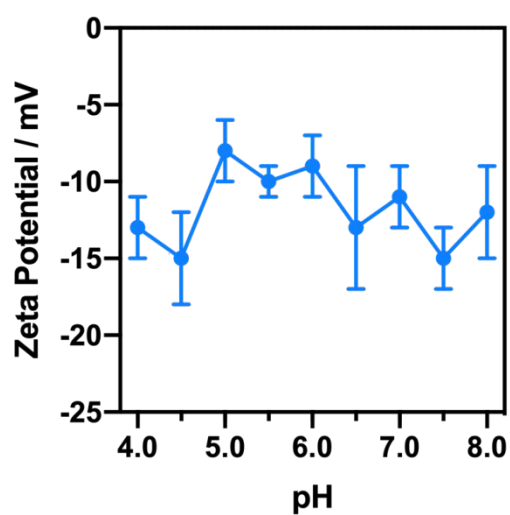


Figure S4. Zeta potentials of D/N-PDA/Hb@HA in the conditions with different pH values.

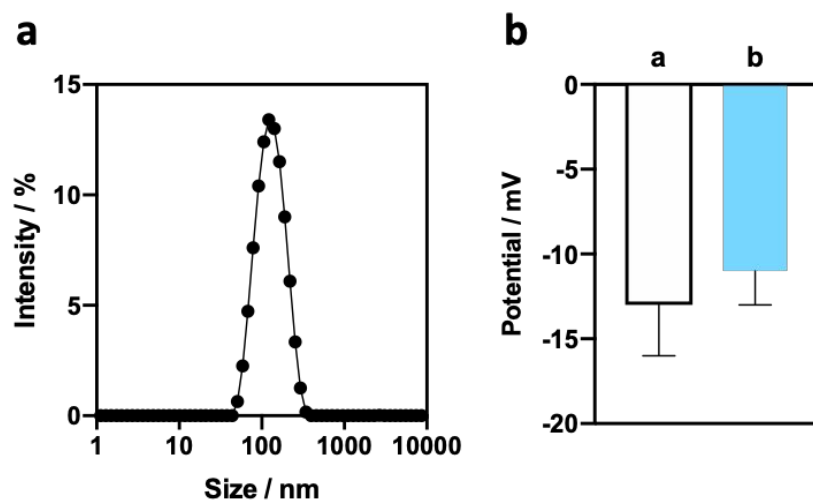


Figure S5. (a) Size distribution of D/N-PDA@HA. (b) Zeta potentials of (a) D/N-PDA@HA and (b) D/N-PDA/Hb@HA.

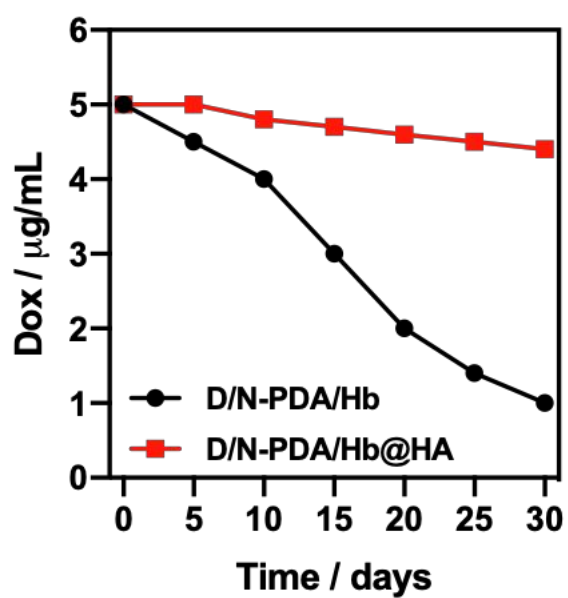


Figure S6. Dox leakage of D/N-PDA/Hb and D/N-PDA/Hb@HA at room temperature for 30 days.

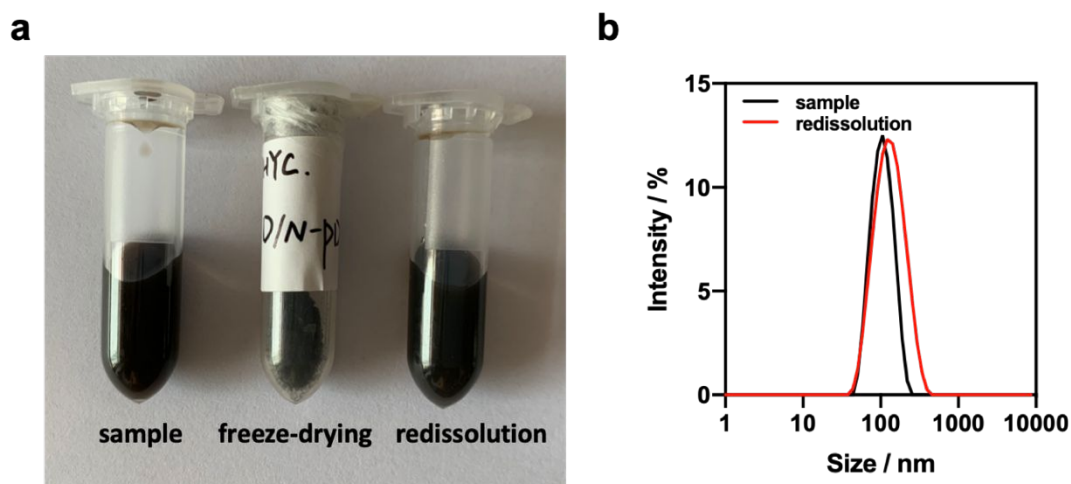


Figure S7. (a) Photographs of freeze-dried powder of D/N-PDA/Hb@HA (sample) and D/N-PDA/Hb@HA with freeze-drying and redissolution. (b) Size distribution of D/N-PDA/Hb@HA and D/N-PDA/Hb@HA with redissolution.

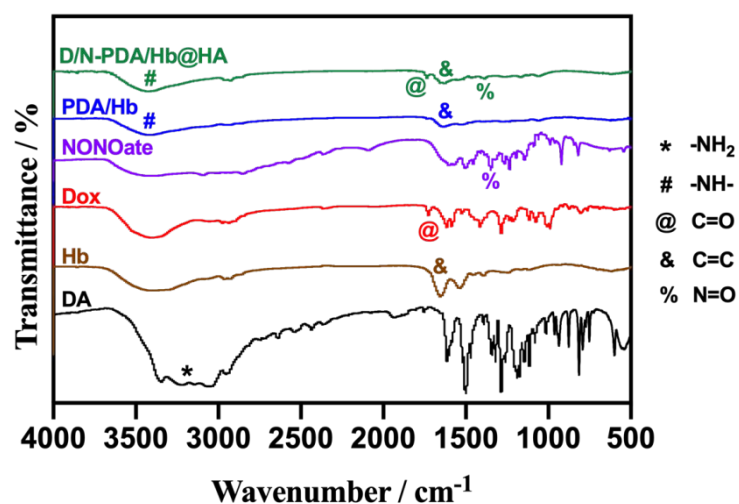


Figure S8. FTIR analysis of DA, Hb, Dox, NONOate, PDA/Hb and D/N-PDA/Hb@HA.

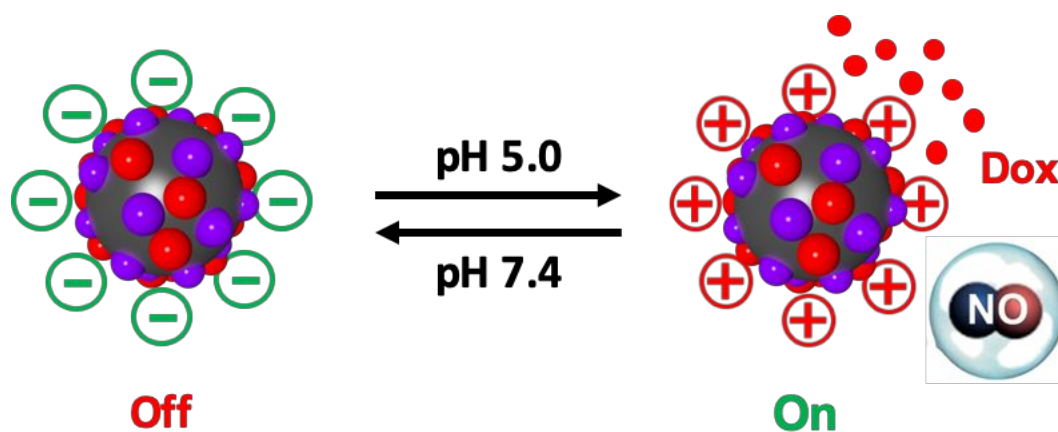


Figure S9. Schematic illustration of Dox release by electrostatic repulsion based on charge reversal and NO release in acidic environment.

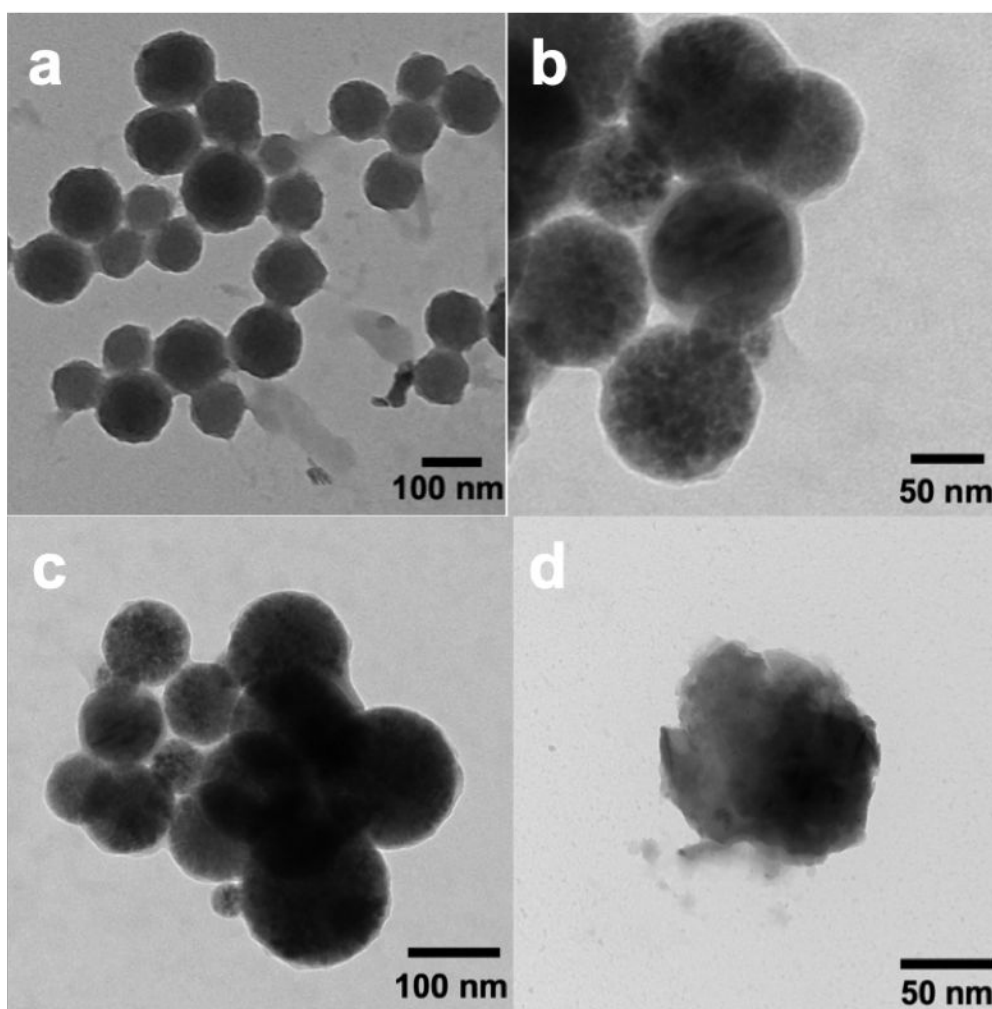


Figure S10. TEM images of D/N-PDA/Hb@HA in (a) pH 7.4, (b) pH 5.5 (first drug release), (c) pH 7.4 (after first drug release) and (d) pH 5.5 (second drug release).

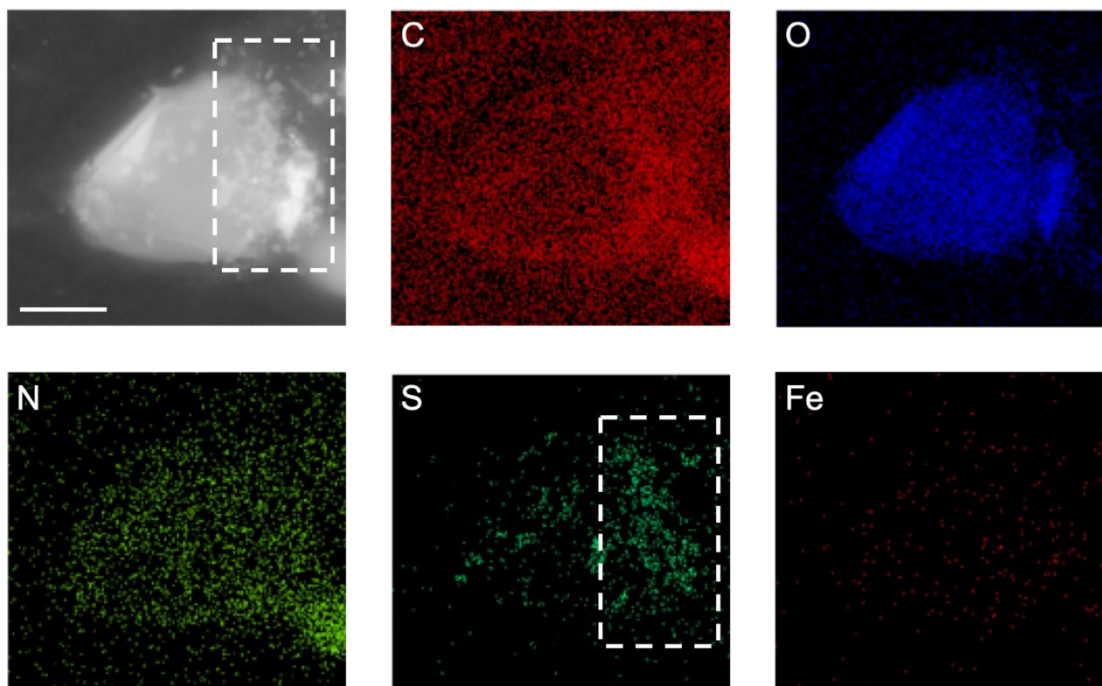


Figure S11. STEM and mapping images of D/N-PDA/Hb@HA with Dox and NO release in the condition of PBS (pH 5.5) (scale bar: 50 nm).

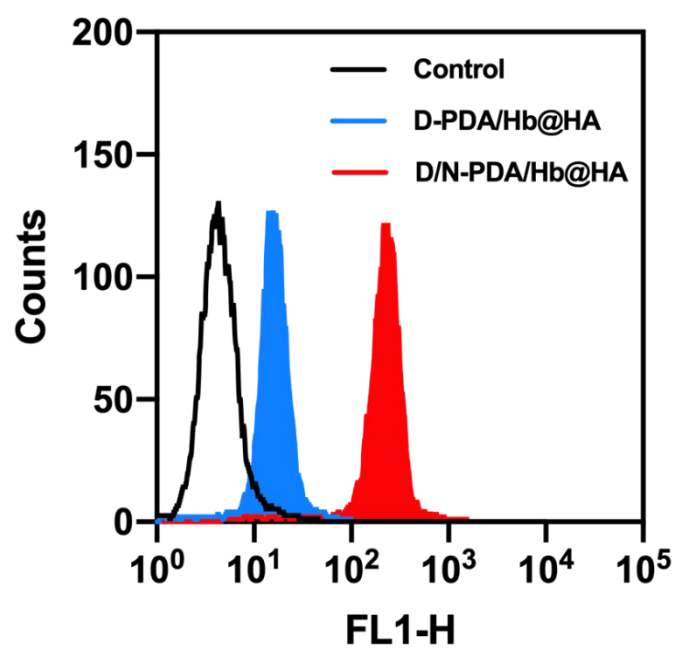


Figure S12. Intracellular fluorescence intensity of LysoTracker Green of PBS as control, D-PDA/Hb@HA and D/N-PDA/Hb@HA by FCM.

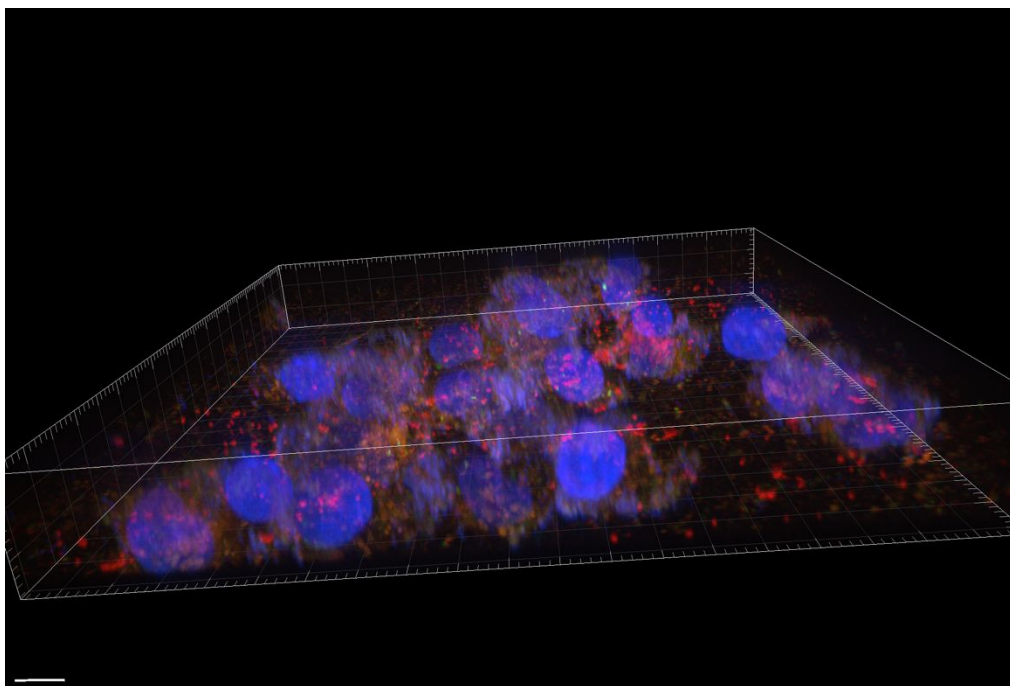


Figure S13. 3d image of HeLa cells treated with D/N-PDA/Hb@HA by CLSM (Blue: cell nucleus, Green: lysosome, Red: D/N-PDA/Hb@HA, Scale bar: 10 μ m).

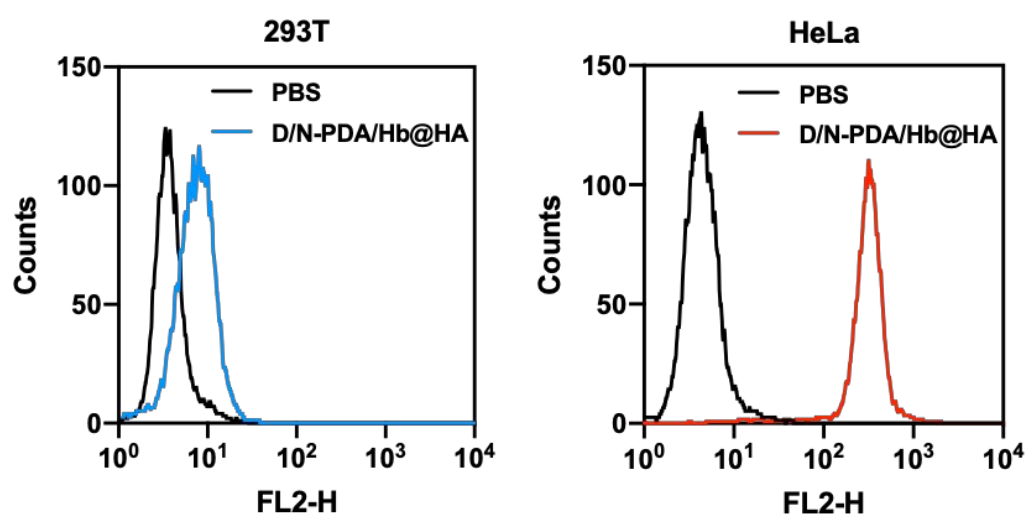


Figure S14. Cellular uptake of 293T and HeLa cells incubated with PBS and D/N-PDA/Hb@HA by FCM analysis.

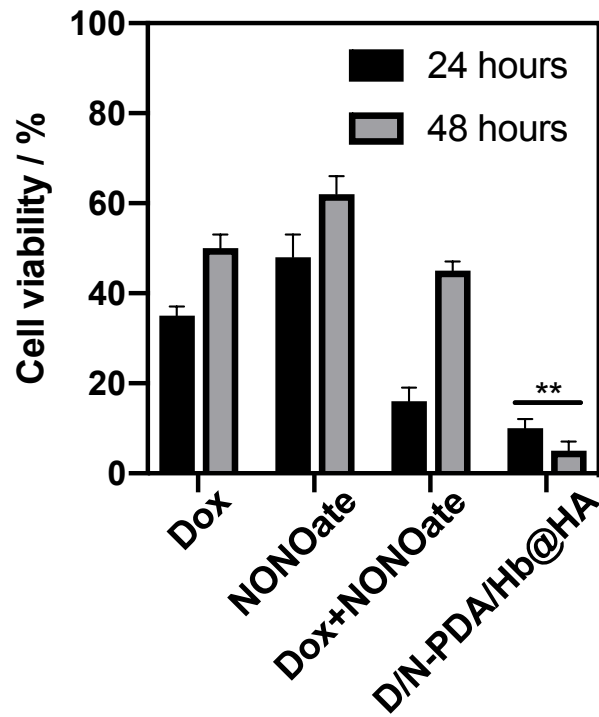


Figure S15. Cytotoxicity of Dox, NONOate, Dox+NONOate and D/N-PDA/Hb@HA on HeLa cells for 24 and 48 hours (** $P < 0.01$ compared with Dox+NONOate).

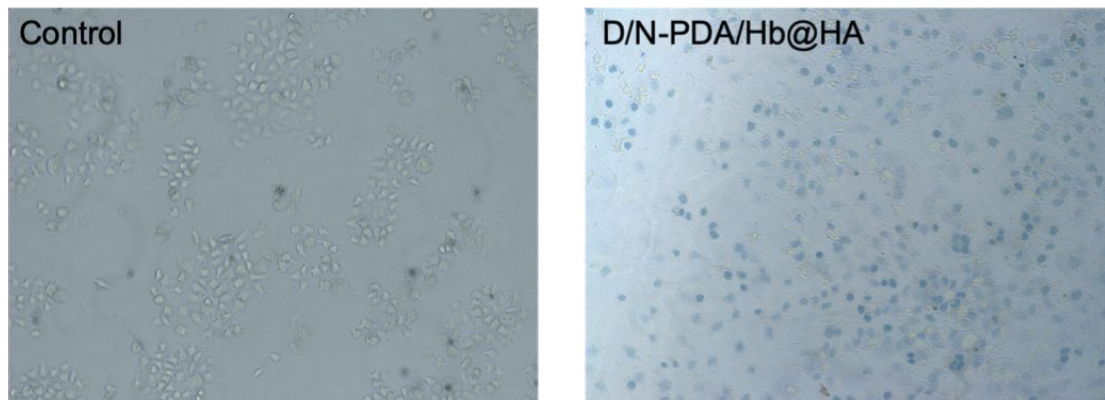


Figure S16. HeLa cells treated with PBS as control and D/N-PDA/Hb@HA by trypan blue staining.

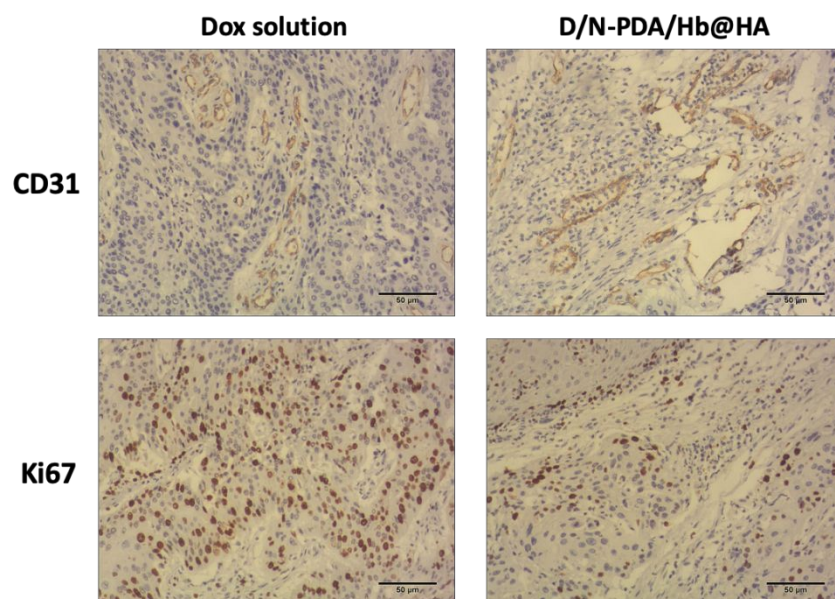


Figure S17. The expression of CD31 (blood vessels) and Ki67 (tumors) of in Dox solution and D/N-PDA/Hb@HA groups using immunohistochemical staining.



Figure S18. Tumor photographs of the mice treated with all tested formulations (Group 1: saline, 2: PDA/Hb, 3: Dox, 4: NONOate, 5: D/N-PDA@HA, 6: D/N-PDA/Hb and 7: D/N-PDA/Hb@HA).

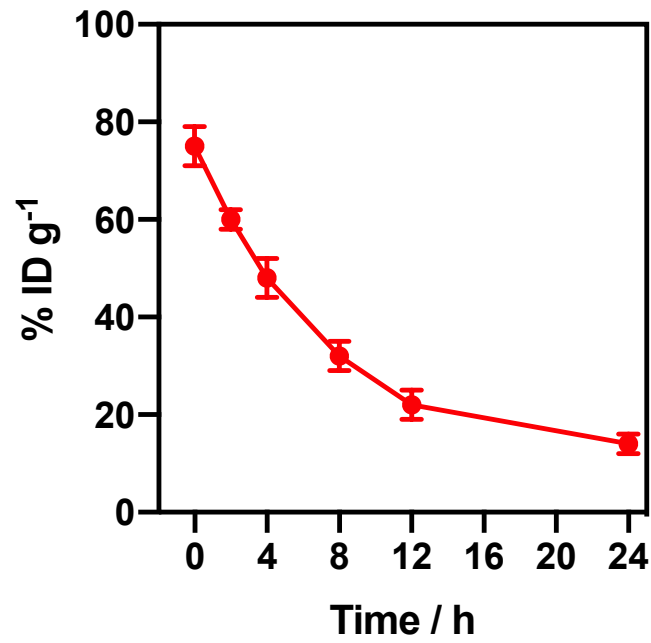


Figure S19. Blood circulation of D/N-PDA/Hb@HA after i.v. injection (%ID g⁻¹ is the percentage of Dox counts per gram of tissue injected).

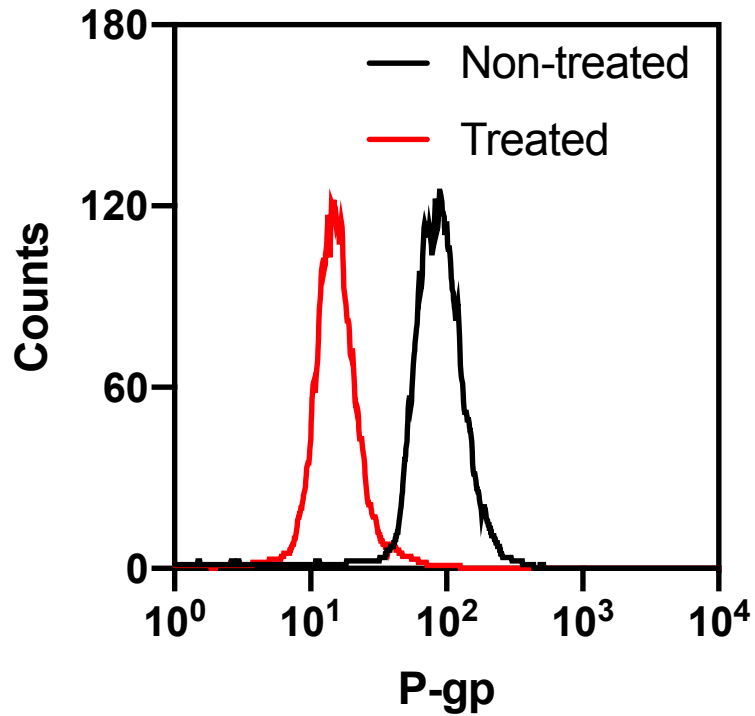


Figure S20. P-gp expression analysis by FCM in treated mice (D/N-PDA/Hb@HA) and non-treated mice.

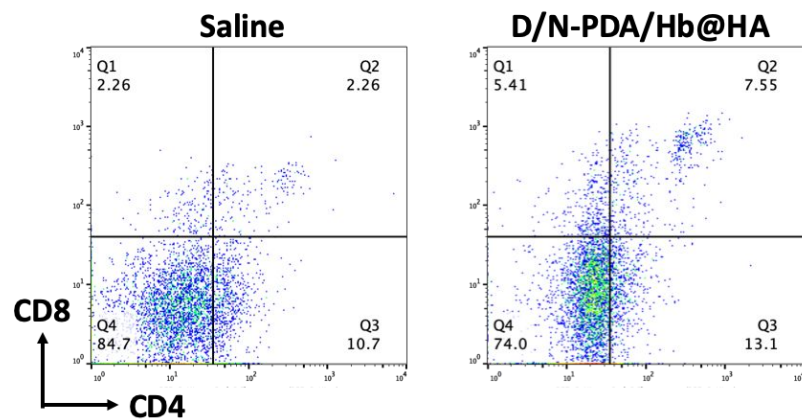


Figure S21. Quantification of CD4 and CD8 T cells in tumors treated with saline and D/N-PDA/Hb@HA.

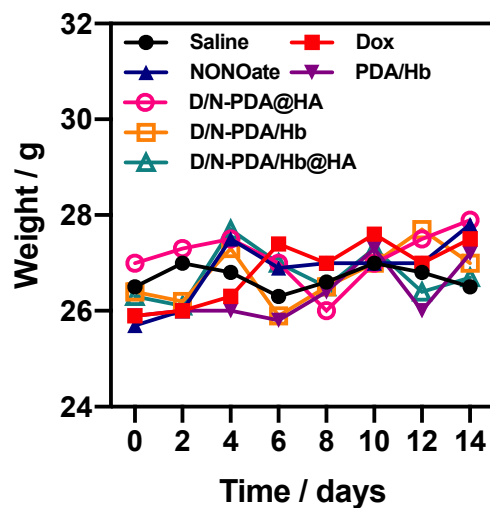


Figure S22. Body weight changes of different groups in the therapeutic period.