Supporting Information for

Vasodilator Hydralazine Promotes Nanoparticle Penetration in Advanced Desmoplastic Tumor

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Supporting Information

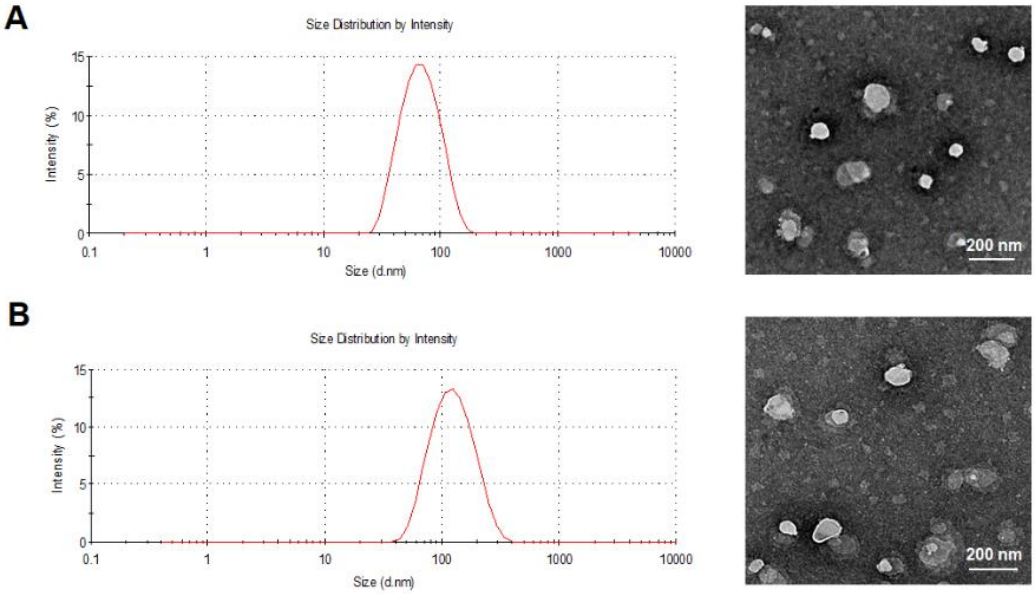


Figure S1. DLS and TEM results of the HDZ-liposomes. DLS and TEM depicting size and morphology of (A) empty-liposomes after extrusion (~75 nm), and (B) HDZ-liposomes after 30 days' storage (~101 nm), respectively.

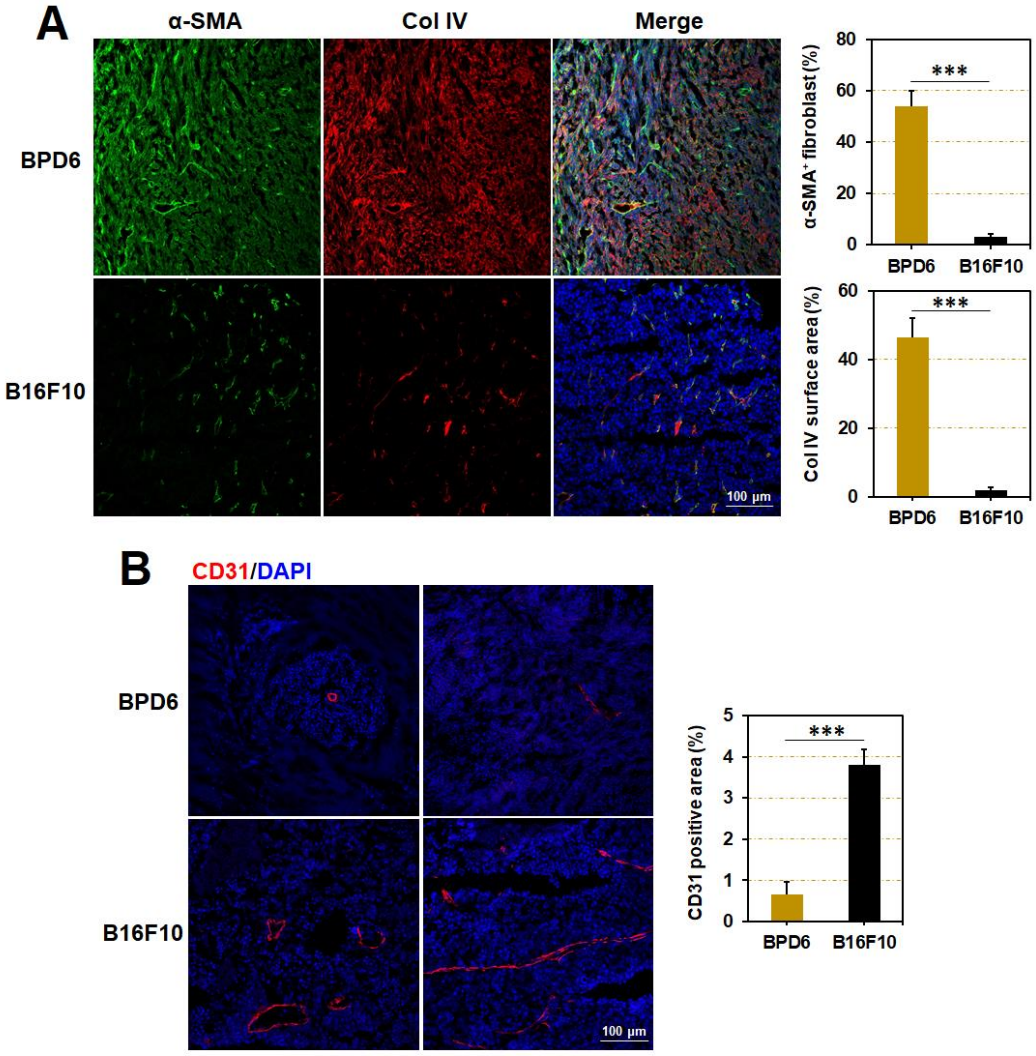
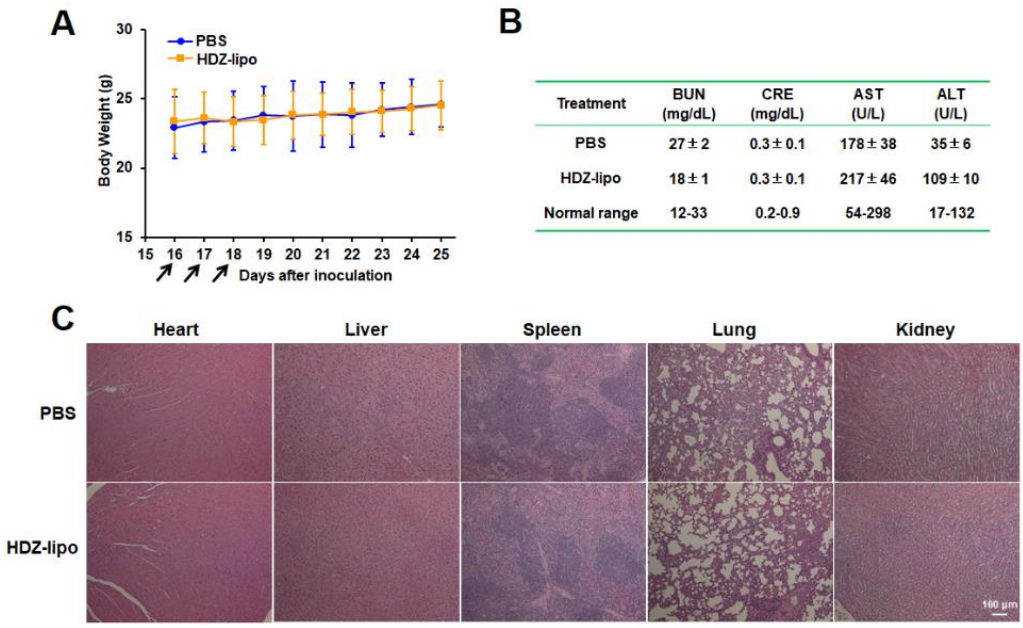


Figure S2. Histological analysis of desmoplastic (BPD6) and wide-type (B16F10) melanoma tumors. (A) Immunostaining of α-SMA (green), collagen IV (red), and DAPI (blue). (B) Immunostaining of CD31 positive (red) vessels. n = 5; *p < 0.05, ** p < 0.01, *** p < 0.001.



Treatment	BUN (mg/dL)	CRE (mg/dL)	AST (U/L)	ALT (U/L)
PBS	27 ± 2	0.3 ± 0.1	178 ± 38	35 ± 6
HDZ-lipo	18 ± 1	0.3 ± 0.1	217 ± 46	109 ± 10
Normal range	12-33	0.2-0.9	54-298	17-132

Figure S3. Toxicity evaluation of HDZ-liposomes. (A) BPD6 tumor-bearing mice body weight changes after three i.v. injections of HDZ-liposomes (black arrows). (B) Serum BUN, CRE, AST and ALT levels. (C) Pathological analyses of heart, liver, spleen, lung and kidney of the BPD6 tumor-bearing mice in the PBS and HDZ-liposome treated groups 7 days post injection.

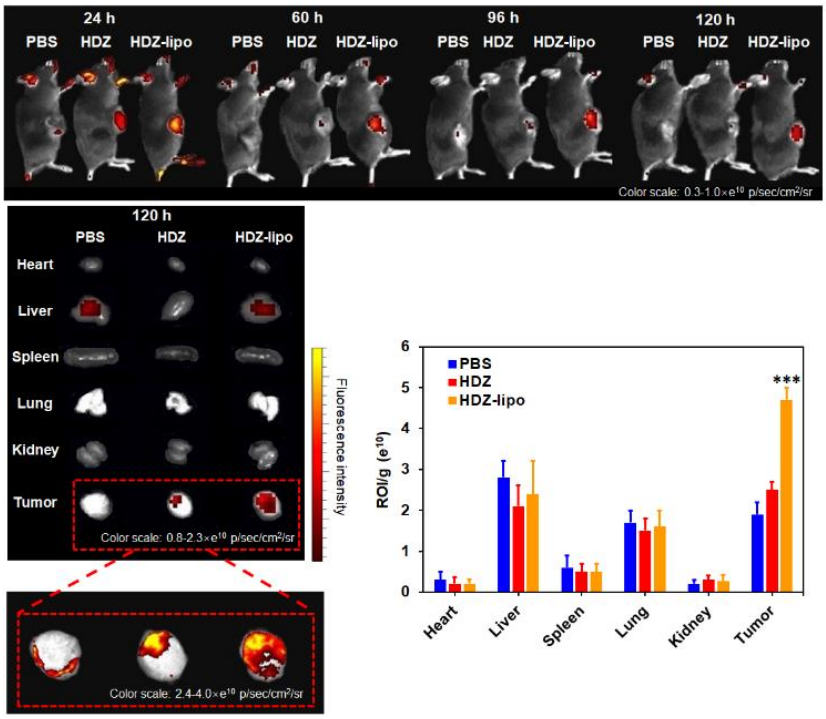


Figure S4. *In vivo* and *ex vivo* images of DiD-loaded liposomes in BPD6 tumor-bearing mice at 24 to 120 h post injection. Mice were pre-treated with PBS, HDZ and HDZ-liposomes, respectively. n = 3 mice per group; *p < 0.05, **p < 0.01, ***p < 0.001, compared to PBS group.

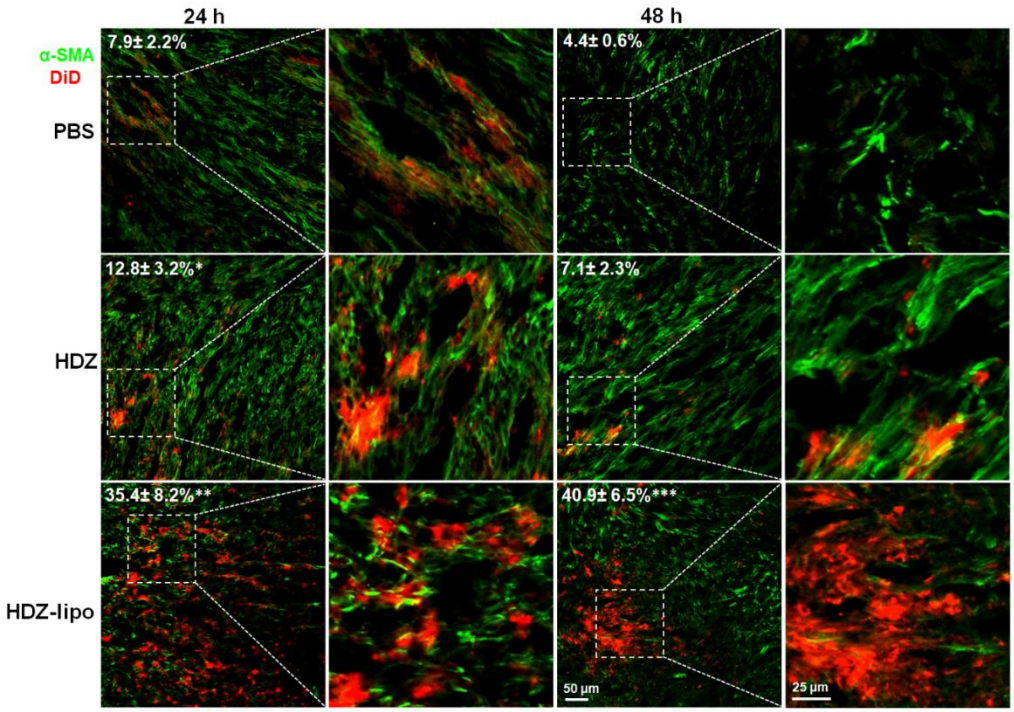


Figure S5. DiD-loaded liposome distribution in TAFs (α-SMA staining) at 24 and 48 h post injection. Mice were pre-treated with PBS, HDZ, and HDZ-liposomes, respectively. n = 5. *p < 0.05, **p < 0.01, ***p < 0.001, compared to PBS group.

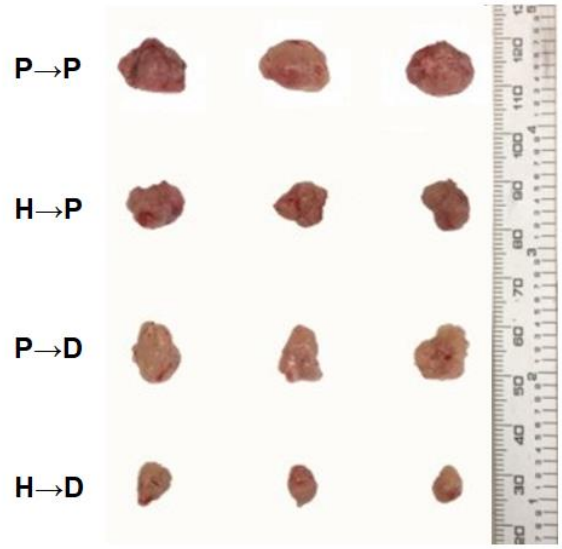


Figure S6. Representative *ex vivo* images of BPD6 tumors after various treatments. PBS followed by PBS (P→P), HDZ-liposomes followed by PBS (H→P), PBS followed by DOX-liposomes (P→D) and HDZ-liposomes followed by DOX-liposomes (H→D).

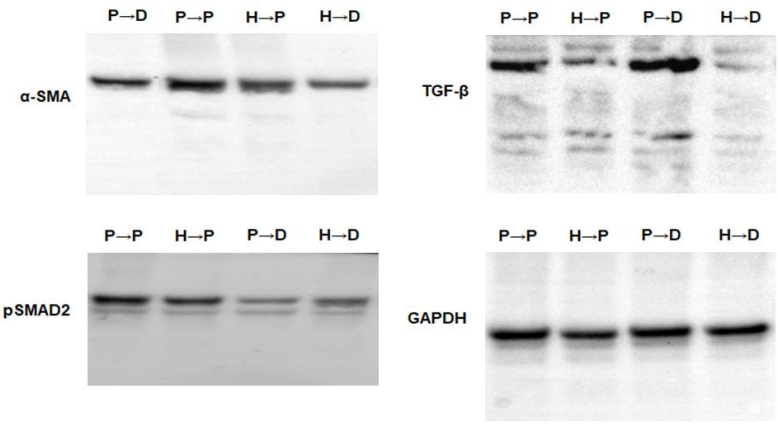


Figure S7. Original photographs of western blotting data in Figure 6E.

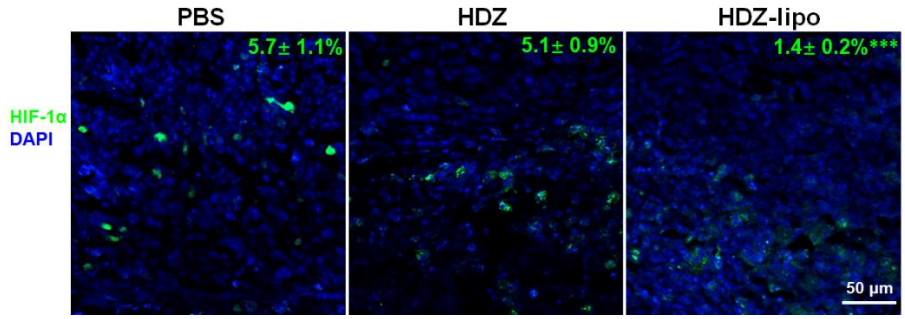


Figure S8. Immunofluorescence staining of HIF-1α in 4T1 tumor after various treatments. n = 5. *p < 0.05, **p < 0.01, ***p < 0.001, compared to PBS group.

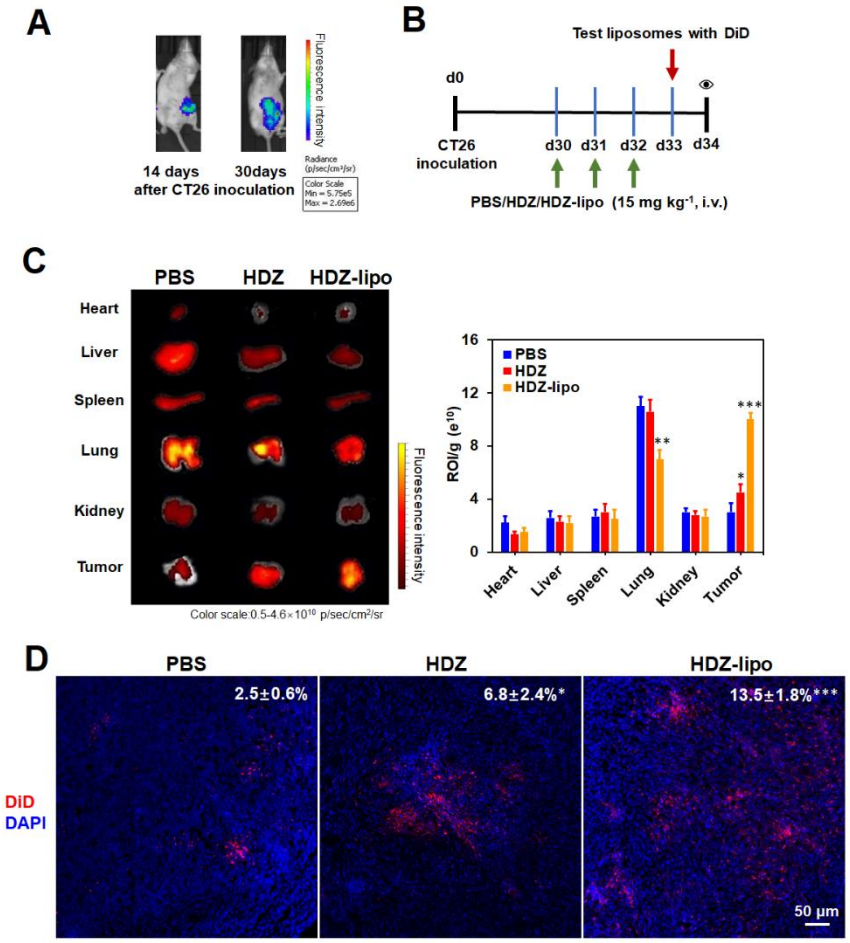


Figure S9. HDZ-liposomes promote the nanoparticle penetration in orthotopic CT26-FL3 tumor model. (A) Advanced orthotopic colorectal tumors establishment on day 30 after CT26-FL3 cells inoculated into the mouse cecum wall. The tumor burden was monitored by bioluminescent analysis. (B) Treatment schedule. (C) *Ex vivo* IVIS image and quantitative analysis of DiD-loaded liposomes in tumor and major organs at 24 h post injection; n = 3 mice per group. (D) Distribution of HDZ-liposomes inside tumor in various treatment groups; n = 5. *p < 0.05, **p < 0.01, ***p < 0.001, compared to PBS group.

Table S1. Antibodies Used in the Study

Antibodies	Company	Catalog	Application
Alexa Fluor®647 Anti-CD3	BioLegend	100209	IF
Alexa Fluor®488 Anti-α-SMA	Invitrogen	4332501	IF
Alexa Fluor®594 Anti-CD31	BioLegend	102432	IF
Anti-collagen IV	Abcam	Ab6586	IF
Anti-fibronectin	Abcam	Ab2413	IF
Anti-HIF-1α	Santa Cruz Biotechnology	SC-10790	IF
Goat Anti-Rabbit IgG, Alexa Fluor®488	Abcam	Ab150077	IF
Goat Anti-Rabbit IgG, Alexa Fluor®594	Abcam	Ab150080	IF
Anti-pSMAD2	Cell signaling	3108S	WB
Anti-α-SMA	Abcam	Ab124964	WB
Anti-TGFβ	Santa Cruz Biotechnology	SC-146	WB
GAPDH	Santa Cruz Biotechnology	SC-25778	WB
Goat anti-rabbit HRP	Abcam	Ab205718	WB
FITC anti-CD8	BioLegend	100705	flow cyt
BV421 anti-CD4	BioLegend	100543	flow cyt
PE/Cy7 anti-CD3	BioLegend	100219	flow cyt
PE/Cy7 anti- NK1.1	BioLegend	552878	flow cyt
PE/Cy7 anti-CD11c	BioLegend	117317	flow cyt
PerCp anti-MHCII	BioLegend	107623	flow cyt
APC/Cy7 anti-CD11b	BioLegend	101225	flow cyt
APC anti-Gr-1	BioLegend	108411	flow cyt
PerCp/Cy5.5 anti-CD206	BioLegend	141715	flow cyt
PE anti-F4/80	BioLegend	123110	flow cyt

Table S2. Primers for real-time PCR used in this study

Primer	Applied Biosystems
Mouse GAPDH	Mm99999915_g1
Mouse CCL2	Mm00441242_m1
Mouse CCL5	Mm01302427_m1
Mouse IL-4	Mm00445259_m1
Mouse IL-10	Mm01288386_m1
Mouse IL-12a	Mm00434169_m1
Mouse IL-1β	Mm00434228_m1
Mouse CXCL9	Mm00434946_m1
Mouse CXCL10	Mm00445235_m1
Mouse CXCL12	Mm00445553_m1
Mouse CXCL13	Mm01208154_g1
Mouse TNF-α	Mm00443260_g1
Mouse IFN-γ	Mm01168134_m1
Mouse TGF-β	Mm01178820_m1