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Supporting information

of

A comparison study to investigate the effect of drug-conjugated site on its delivery efficacy using double hydrophilic block copolymers-based prodrugs

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1. Table S1, S2, S3

2. Figure S1, S2, S3, S4, S5, S6

Table S1. Summary of RAFT-synthesized P(HPMA)-*b*-P(NIPAAm-*st*-EGMA).

	No	Time (min)	Conv. (%) by ^{1}H NMR	Real structure by ^{1}H NMR
NIPPAm:EGMA: [P(HPMA) macro-CTA]:[AIBN] = 462:38:1:1/3 [M]=1.25M T=70 °C MeOH/1,4-dioxane	1	120	26.6	$P(HPMA)_{36}$-*b*-$P(EGMA_{11}$-*st*-$NIPAAm_{123})$
	2	162	44.4	$P(HPMA)_{36}$-*b*-$P(EGMA_{20}$-*st*-$NIPAAm_{205})$
	3	180	51.9	$P(HPMA)_{36}$-*b*-$P(EGMA_{22}$-*st*-$NIPAAm_{240})$

Table S2. Summary of RAFT-synthesized P(HPMA-*st*-EGMA)–*b*-P(NIPAAm).

	No	Time (min)	Conv. (%) by ^{1}H NMR	Real structure by ^{1}H NMR
NIPPAm: [macro-CTA] :[AIBN] =500:1:1/3 [M]=1.25M T=70°C MeOH/1,4-dioxane	1	210	24.0	$P(HPMA_{38}$-*co*-$EGMA_{19})$-*b*-$NIPAAm_{120}$
	2	240	36.0	$P(HPMA_{38}$-*co*-$EGMA_{19})$-*b*-$NIPAAm_{180}$
	3	260	44.4	$P(HPMA_{38}$-*co*-$EGMA_{19})$-*b*-$NIPAAm_{207})$
	4	265	51.9	$P(HPMA_{38}$-*co*-$EGMA_{19})$-*b*-$NIPAAm_{212})$

Table S3. Summary of cell uptake efficiency by semi-quantitative analysis of confocal images using Image J software.

	P(HPMA)-*b*-P(NIPAAm-*st*-(EGMA-DOX))	P(HPMA-*st*-(EGMA-DOX))-*b*-P(NIPAAm)	Free DOX
Cell area ratio (%)	16.5	16.2	19.6

Average fluorescence density per cell area (/pixel)	0.047	0.048	0.037

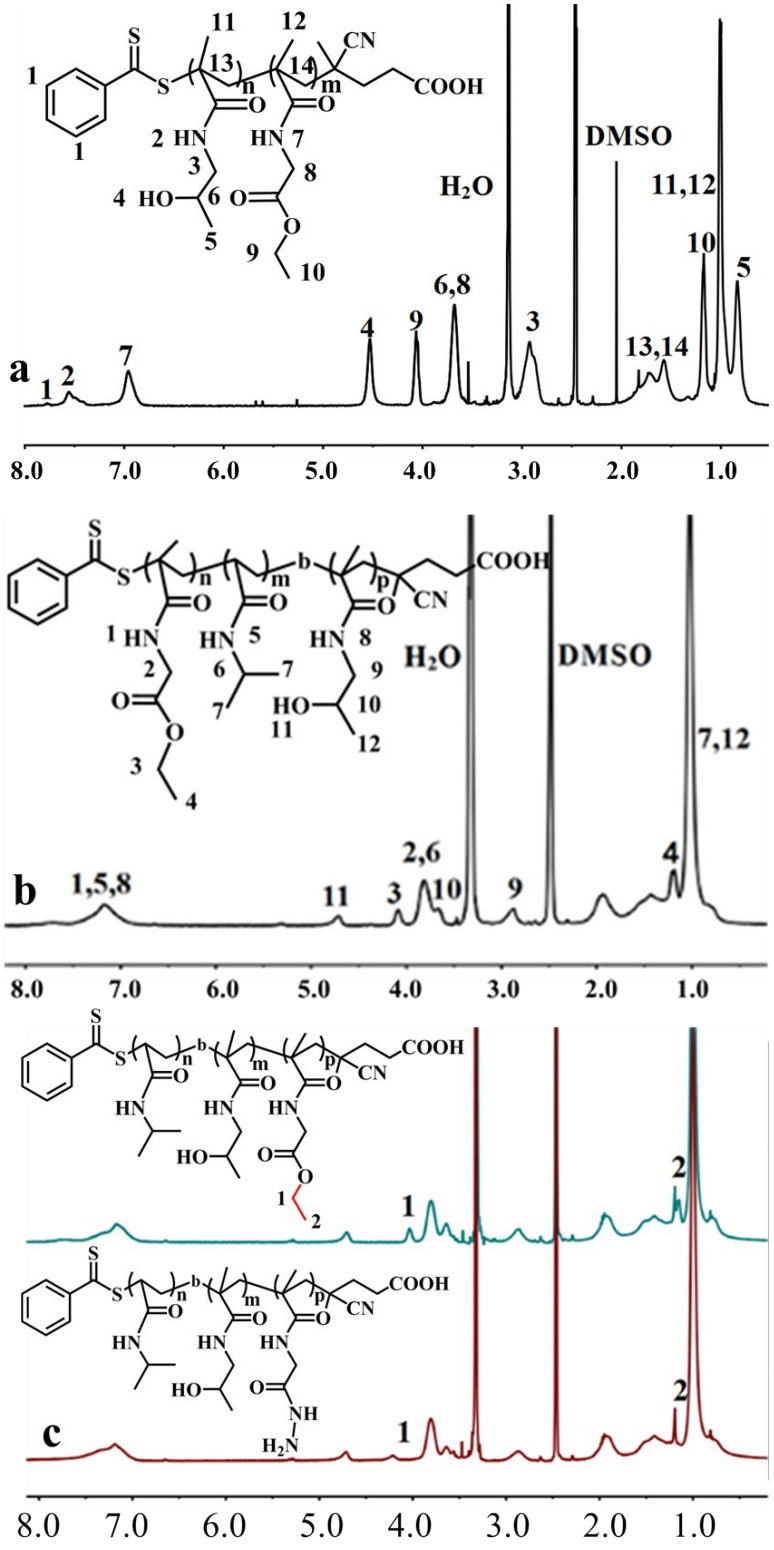

Figure S1. ^{1}H NMR spectra of (a) P(HPMA-*st*-EGMA), (b) P(HPMA-*st*-EGMA)-*b*-P(NIPAAm), and

(c) comparison of P(HPMA)-*b*-P(NIPAAm-*st*-EGMA) and P(HPMA-*st*-(EGMA-hydrazide))-*b*-P(NIPAAm) in d_6-DMSO.

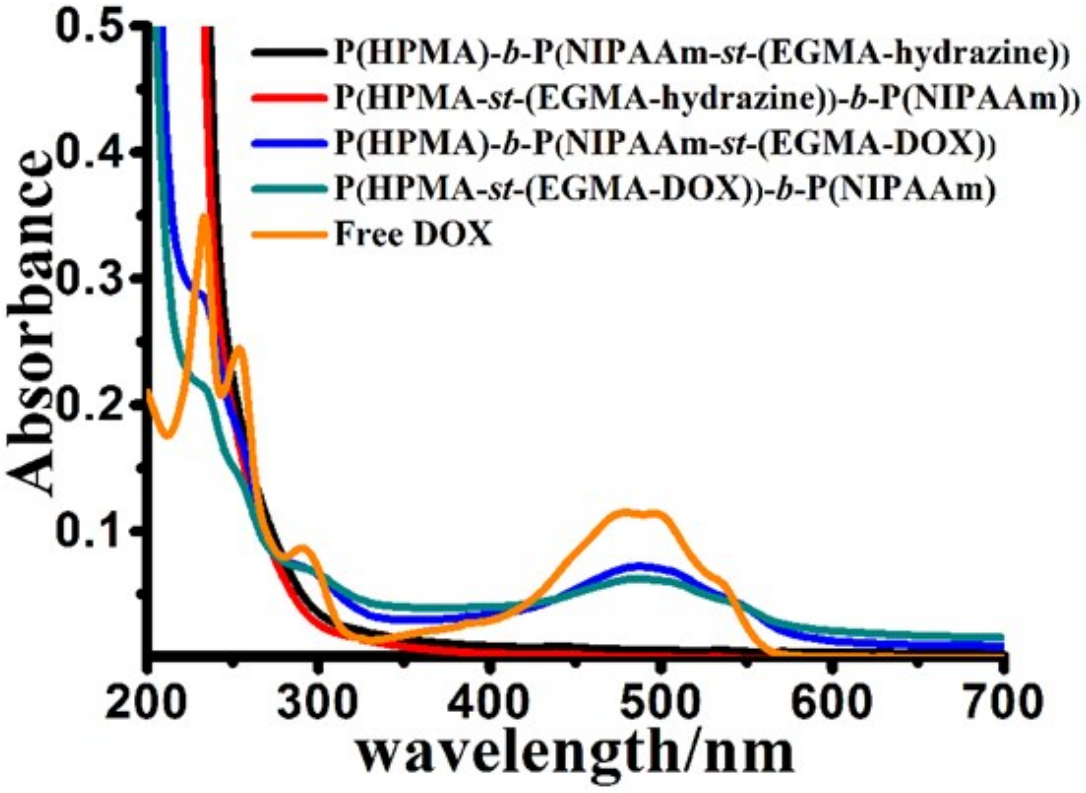

Figure S2. UV-Vis absorbance of P(HPMA)-*b*-(NIPAAm-*st*-(EGMA-hydrazine), P(HPMA-*st*-(EGMA-hydrazine)-*b*-P(NIPAAm) , P(HPMA)-*b*-(NIPAAm-*st*-(EGMA-DOX), P(HPMA-*st*-(EGMA-DOX)-*b*-P(NIPAAm) at 0.033 mg/mL, and free DOX at 0.0006 mg/mL in water.

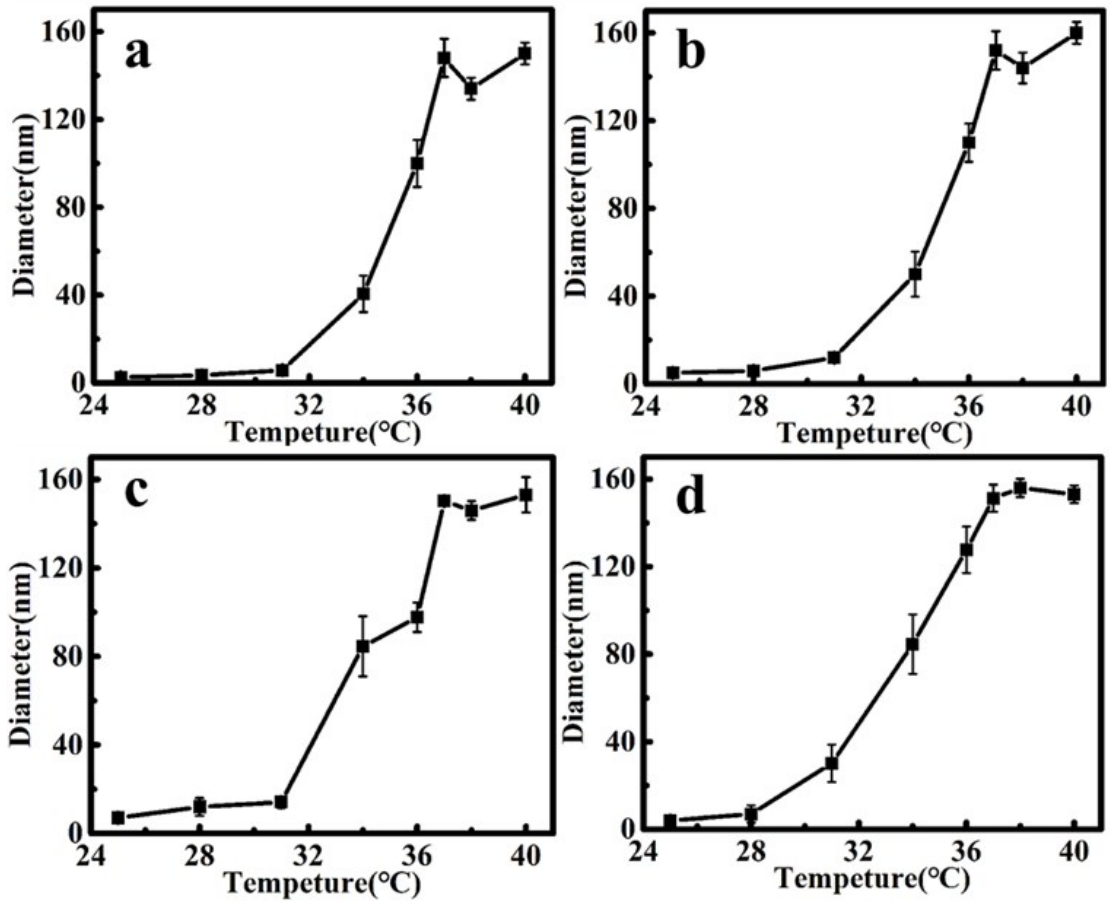

Figure S3. The first and second measurements of temperature-dependent size changes of P(HPMA)-*b*-P(NIPAAm-*st*-(EGMA-DOX)) (a & b) and P(HPMA-*st*-(EGMA-DOX))-*b*-P(NIPAAm) (c & d).

The polymer solution thermostatted at a high temperature was placed into a freezer set at -4°C for the second test immediately upon the completion of the first measurements.

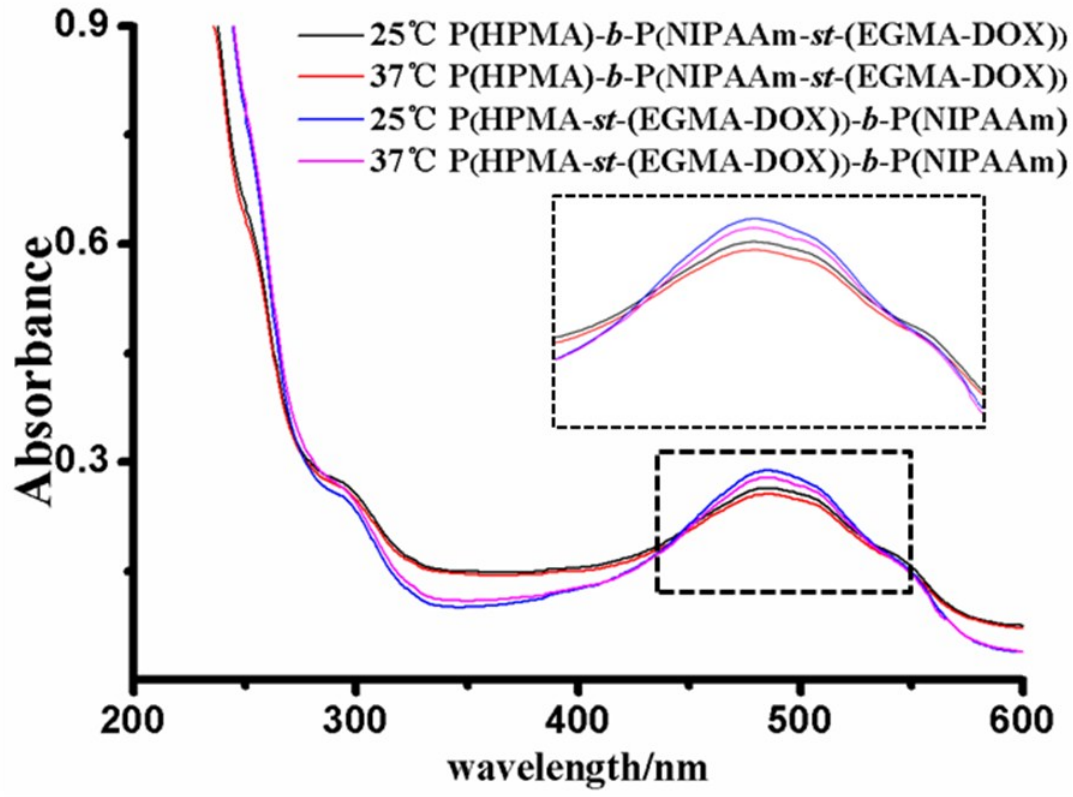

Figure S4. UV-Vis absorbance of P(HPMA)-*b*-(NIPAAm-*st*-(EGMA-DOX) and P(HPMA-*st*-(EGMA-DOX)-*b*-P(NIPAAm) at different temperatures of 25 ^{o}C and 37 ^{o}C in water.

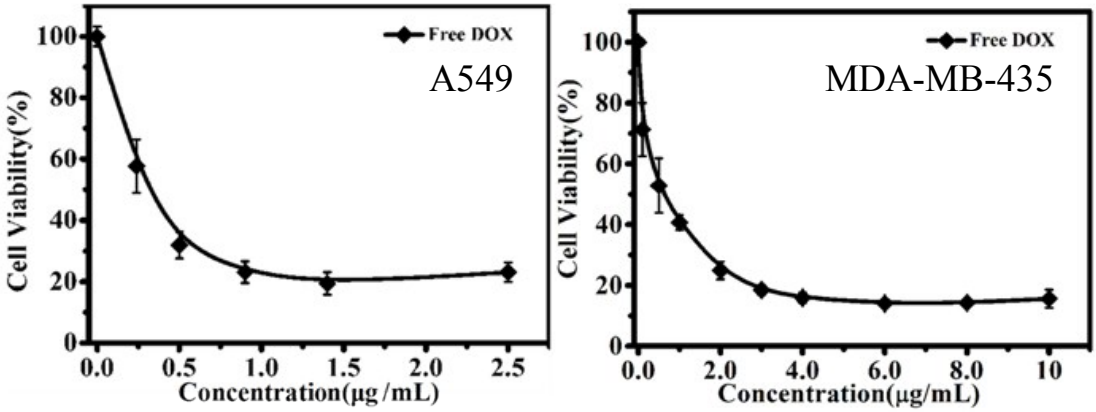

Figure S5. Cell viability of free DOX in A549 and MDA-MB-435 cells evaluated by MTT assay.

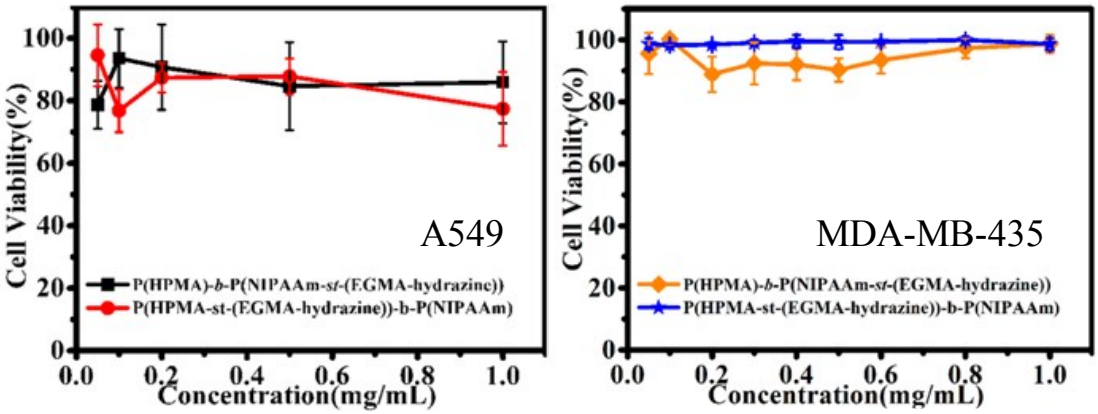

Figure S6. Cell viability of parent DHBCs of P(HPMA)-*b*-P(NIPAAm-*st*-(EGMA-hydrazine)) and P(HPMA-*st*-(EGMA-hydrazine))-*b*-P(NIPAAm) in A549 and MDA-MB-435 cells

evaluated by MTT assay.