Multiple targeting strategies achieve novel protein drug deliver into cancer cells to proapoptosis lung cancer cell by precisely inhibiting survivin

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Supporting Information

Figure legends:

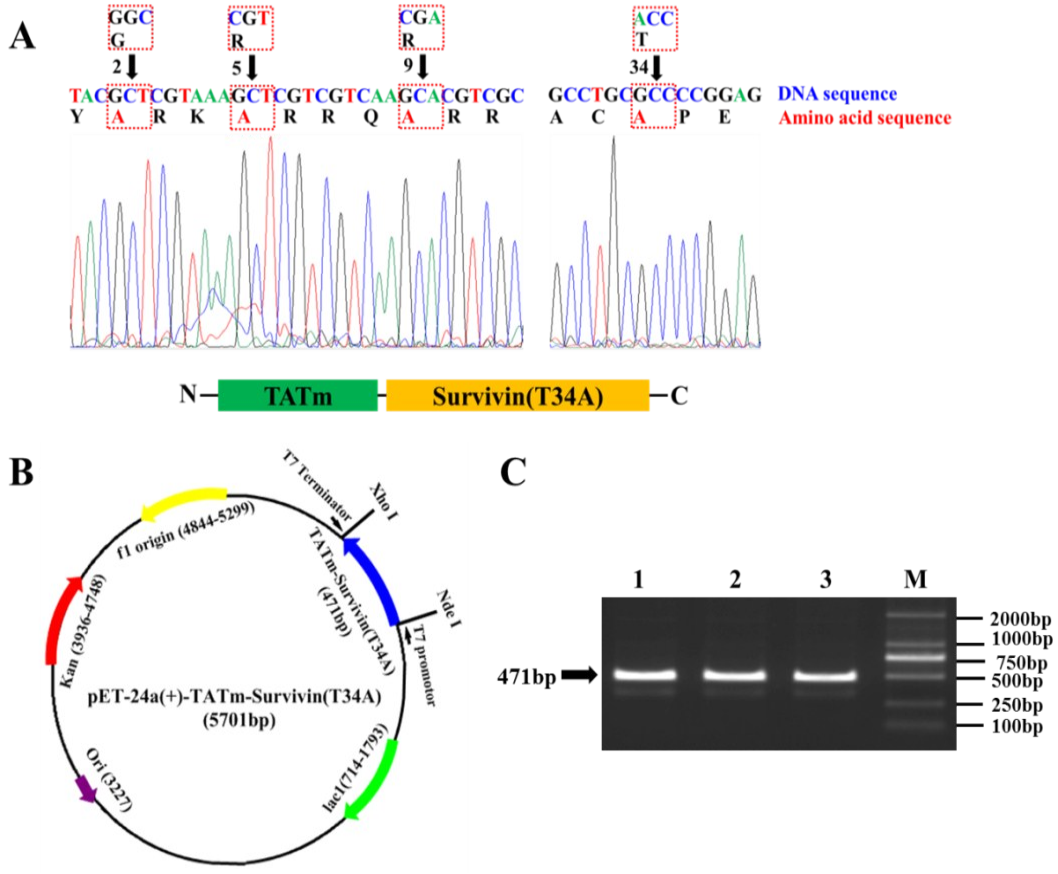

Figure S1. Construction of recombinant plasmid pET-24a(+)-TATm-Survivin (T34A). (A) Results of DNA sequence and amino acid sequence showed G2A, R5A, and R9A mutations in TAT peptide gene and T34A mutation in the survivin gene after PCR amplification. (B) Schematic diagram of the construction of recombinant plasmid pET-24a (+)-TATm-Survivin (T34A). (C) Agarose gel electrophoresis of TATm-Survivin (T34A) *via* PCR amplification. Lane M, DL2000 marker; lane 1-3, TATm-Survivin (T34A) amplification fragment.

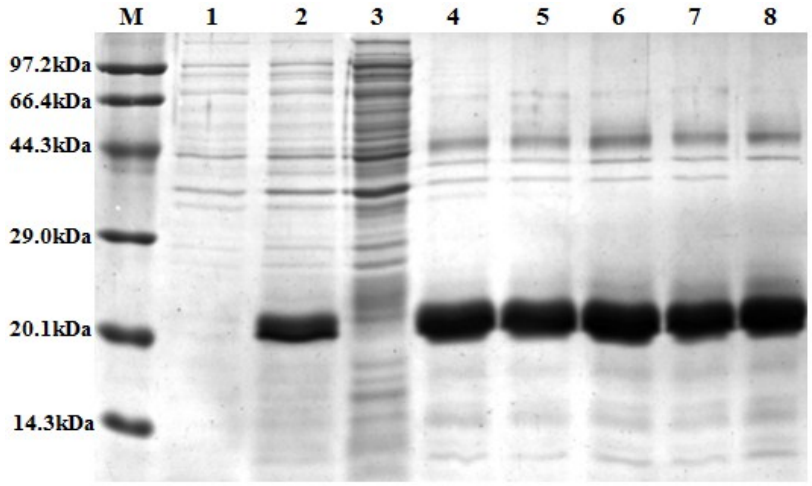

Figure S2. Expression and inclusion body washing of TmSm protein analyzed by SDS-PAGE electrophoresis. Lane M, protein marker; lane 1, whole bacterial solution before induction; lane 2, whole bacterial solution after induction; lane 3, the collected supernatant after cell disruption; lane 4, the collected precipitation after cell disruption; lane 5-7, inclusion body of TmSm protein after 1, 2, and 3 washings, respectively; lane 8, the dissolved inclusion body.

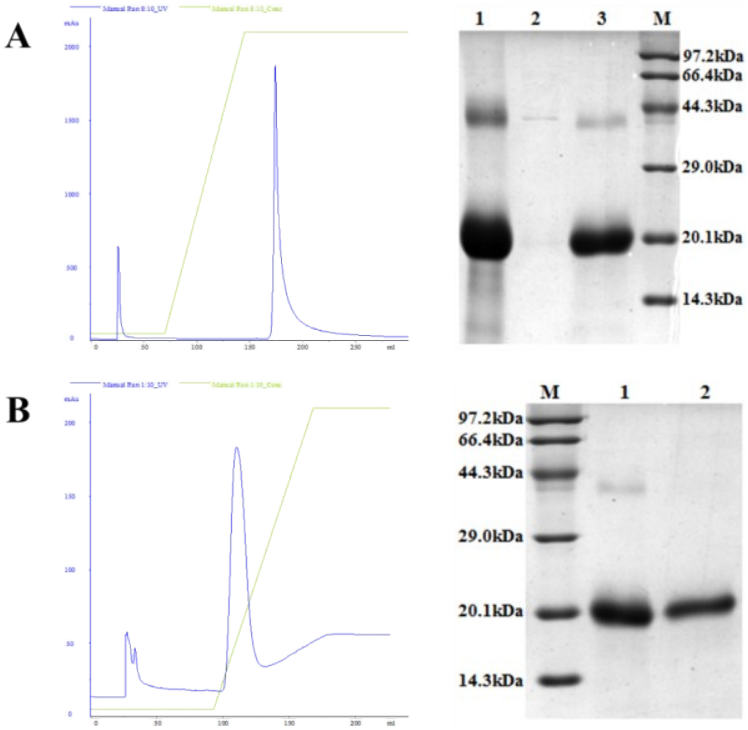

Figure S3. Purification of TmSm protein and SDS-PAGE analysis. (A) On-column refolding of TmSm protein *via* SP sepharose chromatogram and SDS-PAGE analysis. Lane M, protein marker; lane 1, the dissolved inclusion body; lane 2, column filtrate; lane 3, SP column eluate. (B) Purification of TmSm protein *via* nickel column chromatogram and SDS-PAGE analysis. Lane M, protein marker; lane 1, SP column eluate; lane 2, nickel column eluate.

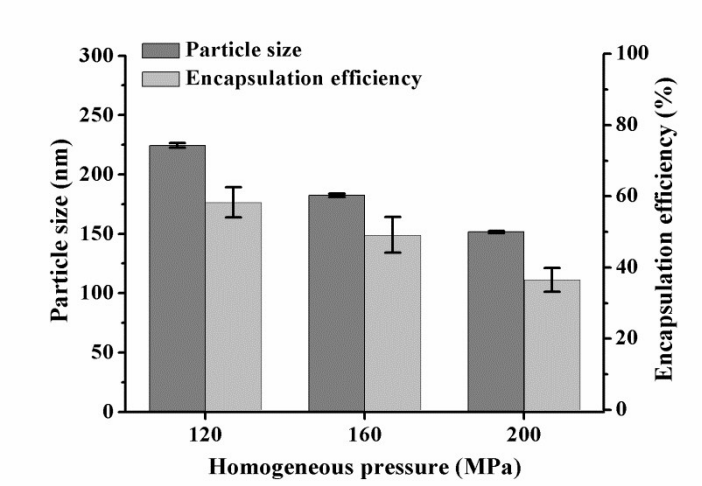

Figure S4. Particle size and EE of TmSm/PLGA NPs produced by high-pressure homogenization with 120, 160, and 200 MPa for 1 cycle, respectively. Data were expressed as mean ± SD (n = 3).

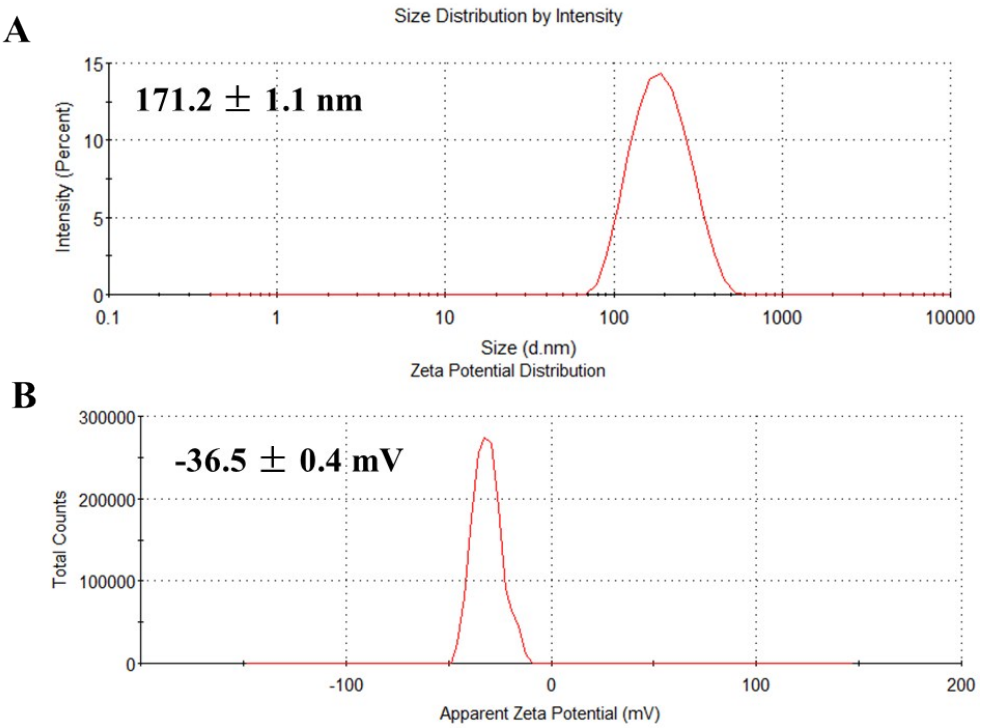

Figure S5. Particle size (A) and zeta potential (B) of blank PLGA NPs without PEGylation. Data were expressed as mean ± SD (n = 3).

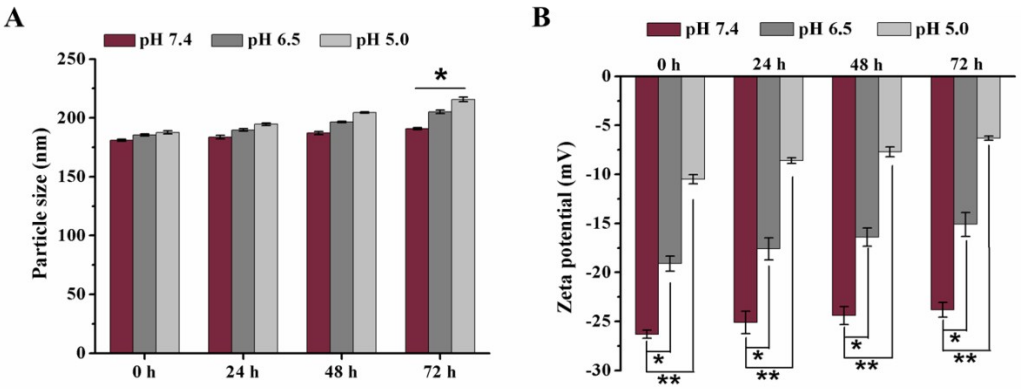

Figure S6. Particle size (A) and zeta potential (B) of TmSm/PLGA NPs after incubation with different pH media for 24, 48, and 72 h, respectively. Data were expressed as mean ± SD (n = 3). *P < 0.05 and **P < 0.01.

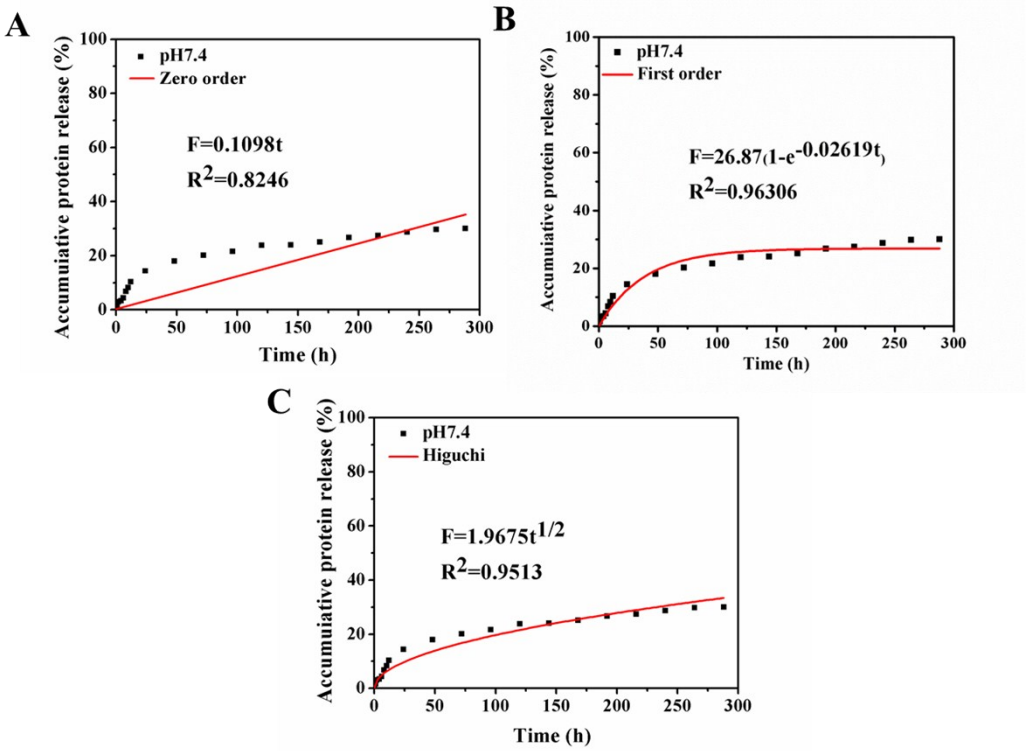

Figure S7. Release kinetics plots for TmSm from PLGA NPs were evaluated by (A) Zero-order, (B) First-order, and (C) Higuchi models, respectively.

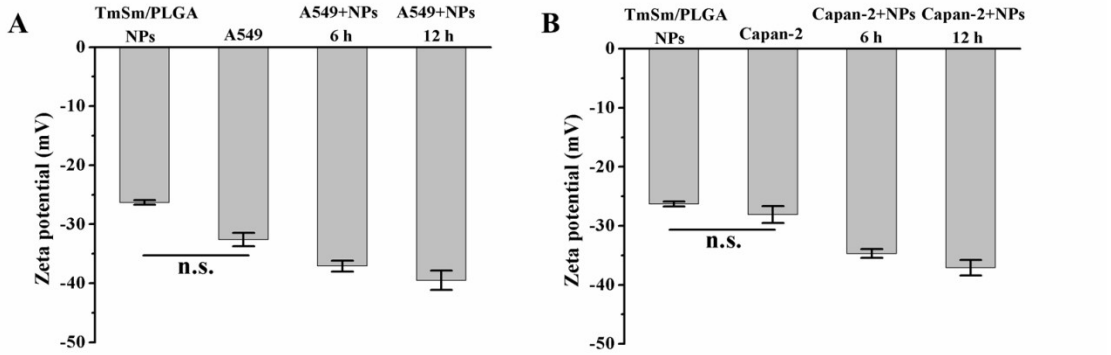

Figure S8. Zeta potential change of A549 cells (A) and Capan-2 cells (B) after incubated with TmSm/PLGA NPs for 6 and 12 h, respectively. Data were expressed as mean ± SD (n = 3). n.s. represented not significant.

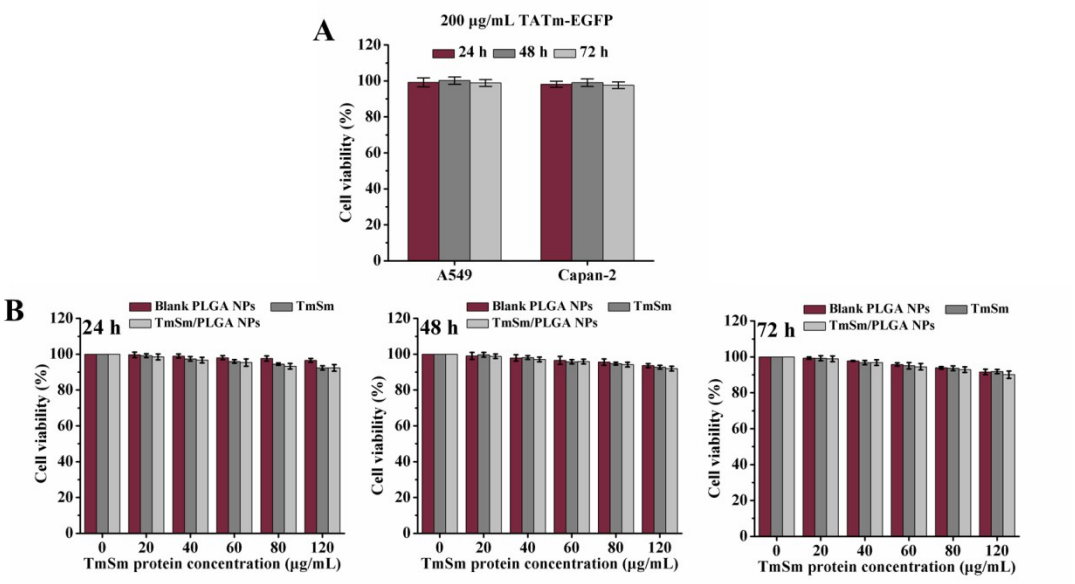

Figure S9. Cytotoxicity assay. (A) The viabilities of A549 and Capan-2 cells treated with TATm-EGFP protein at 200 μg/mL (equivalent to the maximum molar concentration of TmSm) for 24, 48, and 72 h, respectively. (B) L-02 cells were incubated with TmSm, blank PLGA NPs, and TmSm/PLGA NPs for 24, 48, and 72 h, respectively. Data were expressed as mean ± SD (n = 3).

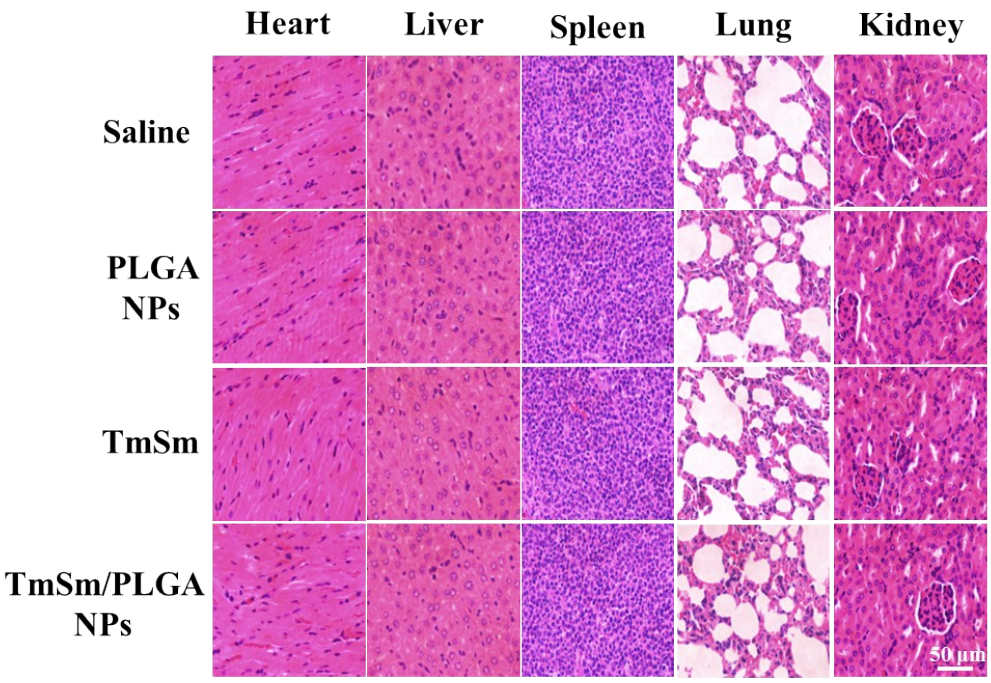

Figure S10. Histological examination of major organs. After A549 tumor-bearing nude mice were treated with saline, TmSm, blank PLGA NPs, and TmSm/PLGA NPs for 15 days, major organs (heart, liver, spleen, lung, and kidney) were collected and fixed with 4% paraformaldehyde for paraffin slicing, followed by HE staining for histological examination. All the images were taken at 400× magnification. (Bar = 50 μm)

Tables:

Table S1. Mathematical models for the release of TmSm from mPEG-PLGA NPs.

Release profile	Parameter	Zero-order	First-order	Higuchi	Ritger-Peppas	Weibull
pH 7.4	R^2	0.8246	0.9631	0.9513	**0.9814**	**0.9915**
	k	0.1098	0.0262	1.9675	4.8601	0.0435
	n	/	26.8700	/	0.3397	32.8654
	m	/	/	/	2.1451	1.5410
pH 6.5	R^2	0.6672	0.9014	0.4221	**0.9789**	**0.9944**
	k	32.774	0.1054	4.8176	23.4478	0.3957
	n	/	59.5617	/	0.1932	69.5489
	m	/	/	/	-1.8248	3.4846
pH 5.0	R^2	0.7018	0.9174	0.6090	**0.9786**	**0.9956**
	k	36.4848	0.0799	6.2770	27.1000	0.4413
	n	/	78.7931	/	0.2191	91.9163
	m	/	/	/	-3.0627	4.4870