Supporting data

The pH-Triggered Triblock Nanocarrier Enabled Highly Efficient siRNA Delivery for Cancer Therapy

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Table S1. Detailed sequences of siRNAs in this research work

Name (siRNA)[a)]	Sequence (5′-3′)[b)]	Target gene
siNC	(s): CCUUGAGGCAUACUUCAAAdTdT (as): UUUGAAGUAUGCCUCAAGGdTdT	*none*
siFL	(s): CCCUAUUCUCCUUCUUCGCdTdT (as): GCGAAGAAGGAGAAUAGGGdTdT	*luciferase*
h-siPLK1	(s): UGAAGAAGAUCACCCUCCUUAdTdT (as): UAAGGAGGGUGAUCUUCUUCAdTdT	*plk1*
m-siApoB	(s): GUCAUCACACUGAAUACCAAUdTdT (as): AUUGGUAUUCAGUGUGAUGACACdTdT	*apoB*
m-siSCD1 h-siRRM2	Designed and protected by Suzhou Ribo Life Science Co., Ltd. (Suzhou, Jiangsu Province, China)	*scd1* *rrm2*

[a)] siNC and Cy5-siRNA shared the same sequence. The letters h and m before dash represent human source and mouse source, respectively; [b)] The letters s and as in brackets represent sense strand and antisense strand, respectively.

Table S2. Detailed sequences of primers in this research work

Name (primer)[c)]	Sequence (5′-3′)[d)]	Target gene
h-GAPDH	(f): AGAAGGCTGGGGCTCATTTG (r): AGGGGCCATCCACAGTCTTC	*gapdh*
m-*β*-Actin	(f): AGCCATGTACGTAGCCATCC (r): CTCTCAGCTGTGGTGGTGAA	*actin*
h-PLK1	(f): GCCCCTCACAGTCCTCAATA (r): TACCCAAGGCCGTACTTGTC	*plk1*
m-ApoB	(f): TTCCAGCCATGGGCAACTTTACCT (r): TACTGCAGGGCGTCAGTGACAAAT	*apoB*
m-SCD1	(f): TGGTGAACAGTGCCGCGCAT (r): ACTCAGAAGCCCAAAGCTCAGCTAC	*scd1*

[c)] The letters h and m before dash represent human source and mouse source, respectively; [d)] The letters f and r in brackets represent forward primer and reverse primer, respectively.

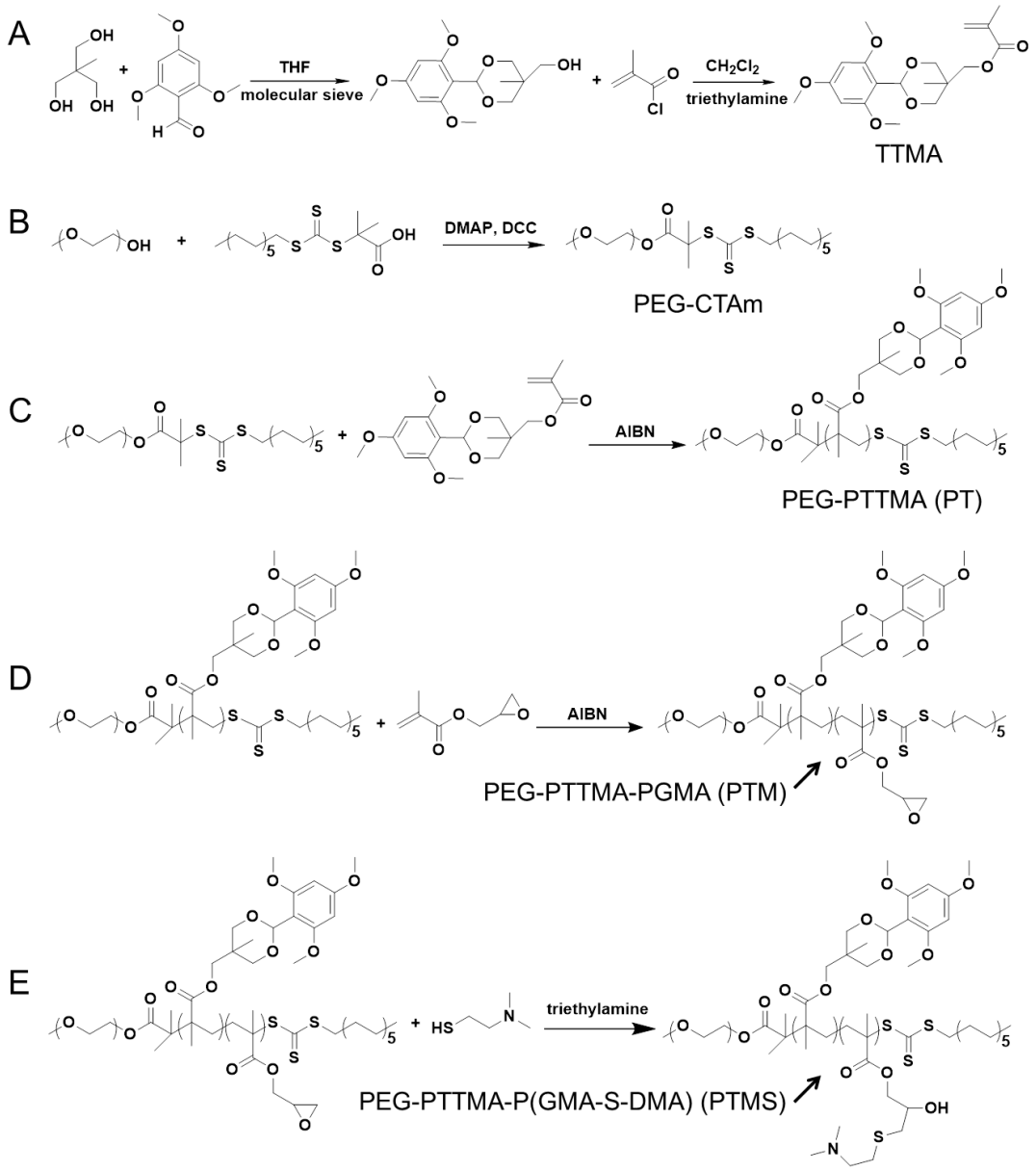

Fig. S1. Synthesis routes of TTMA, PEG-CTAm, PEG-PTTMA (PT), PEG-PTTMA-PGMA (PTM) and PEG-PTTMA-P(GMA-S-DMA) (PTMS) polymers.

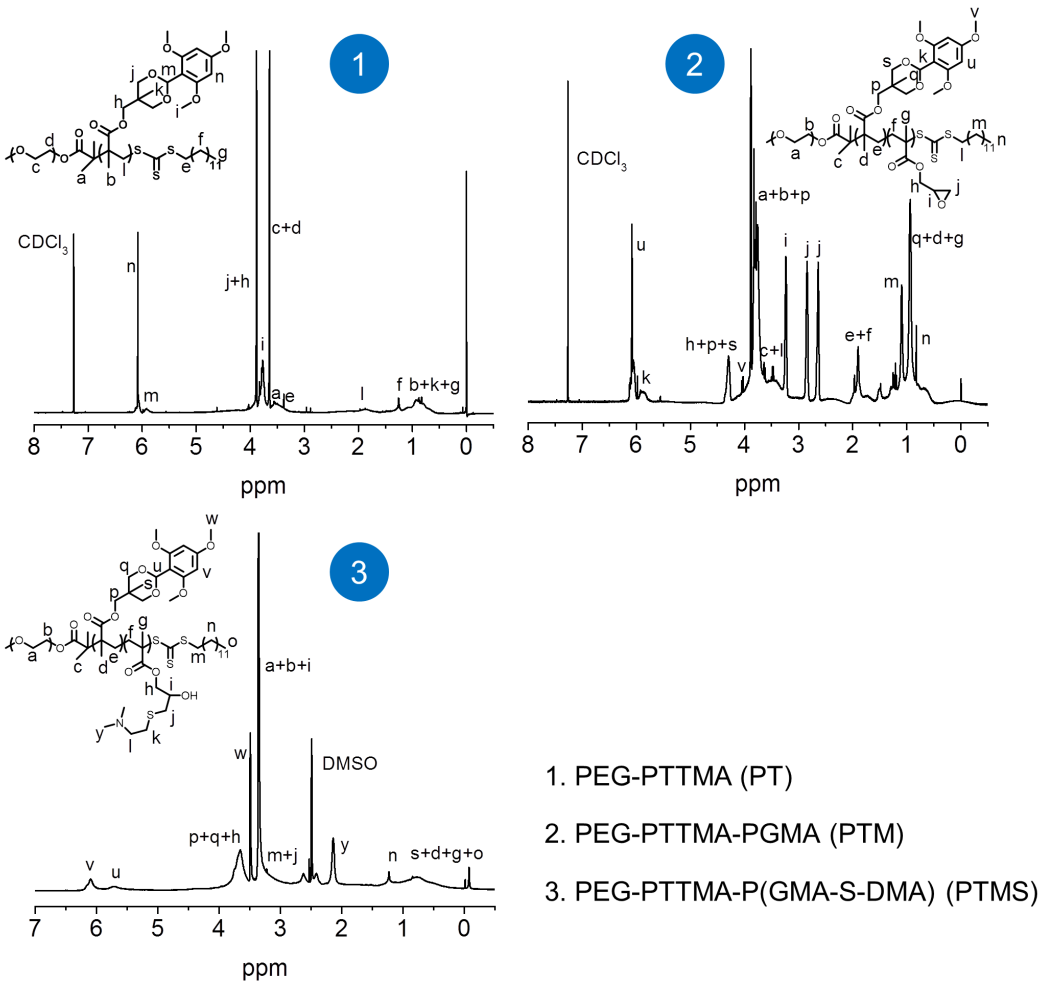

Fig. S2. The H^1-NMR spectra of PEG-PTTMA (PT), PEG-PTTMA-PGMA (PTM) and PEG-PTTMA-P(GMA-S-DMA) (PTMS) polymers.

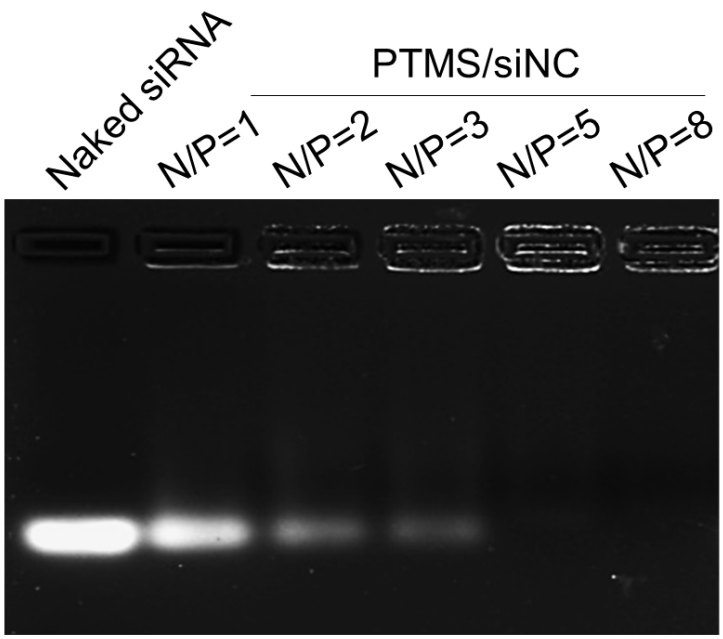

Fig. S3. Gel retardation measurement of PTMS/siNC complexes. Agarose gel electrophoresis was performed with N/P ratios of 1:1, 2:1, 3:1, 5:1 and 8:1 at the fixed amount of 0.3μg siNC.

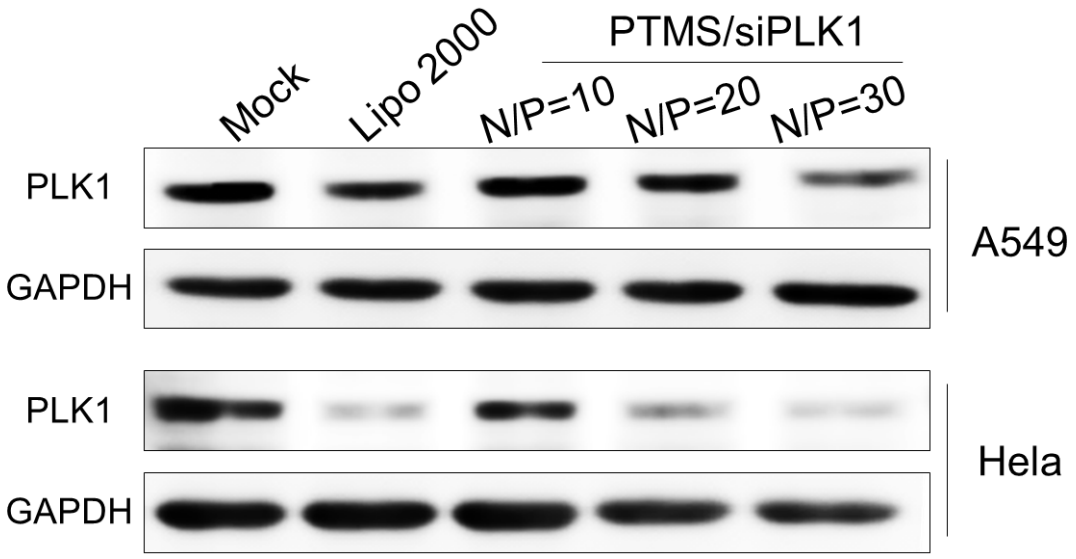

Fig. S4. *In vitro* gene silencing effect of PTMS/siPLK1 formulations. After treatment for 48h at the final siRNA transfection concentration of 50nM, western blot was performed to measure silencing effects in A549 and Hela cells.

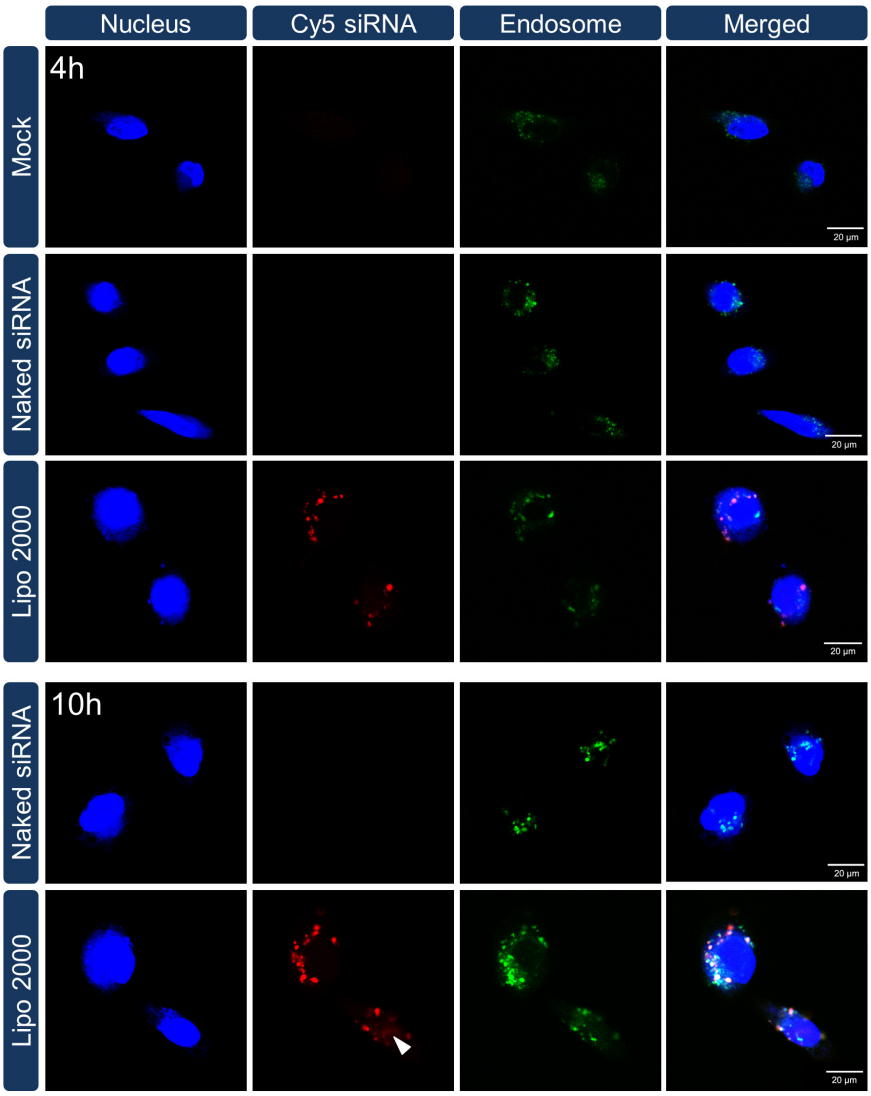

Fig. S5. Subcellular localization study of polyplexes. Confocal images of MDA-MB-231 cells transfected by various formulations for 4h and 10h at the final siRNA concentration of 50nM. From left to right, nucleus was stained by DAPI (blue), siRNA was labeled by Cy5 (red), endosomes/lysosomes were stained by lysotracker green (green) and merged images. Scale bar is 20μm, white arrow indicates the diffused Cy5 signal.

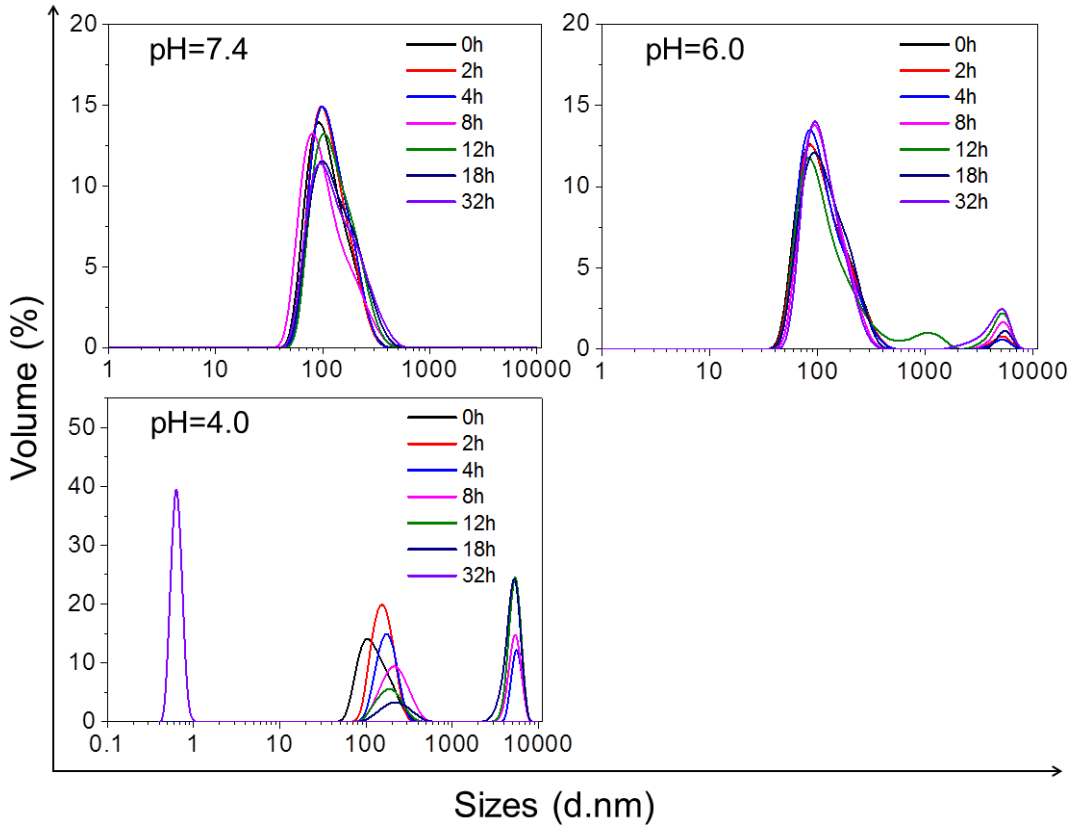

Fig. S6. Size changes of PTMS NPs in acidic buffer. The pH of PTMS NPs solution (10μg/ml) was adjusted to 7.4, 6.0, and 4.0, respectively, shaking at 37℃. Size changes were recorded and analyzed by DLS in following 32h. Size were calculated by volume-weighted.

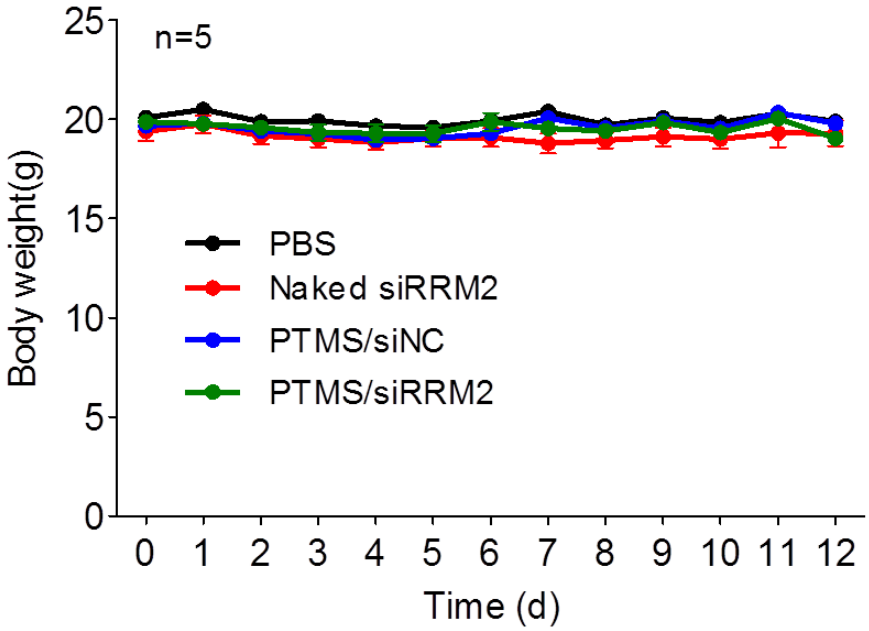

Fig. S7. Body weight changes of nude BALB/c mice. To evaluate tumor growth inhibition of PTMS/siRRM2 in vivo. Tumor-bearing mice were treated every three days for 4 times. Body weight was recorded every day post dosing until day 12 to monitor the side effects of various formulations (n=5).

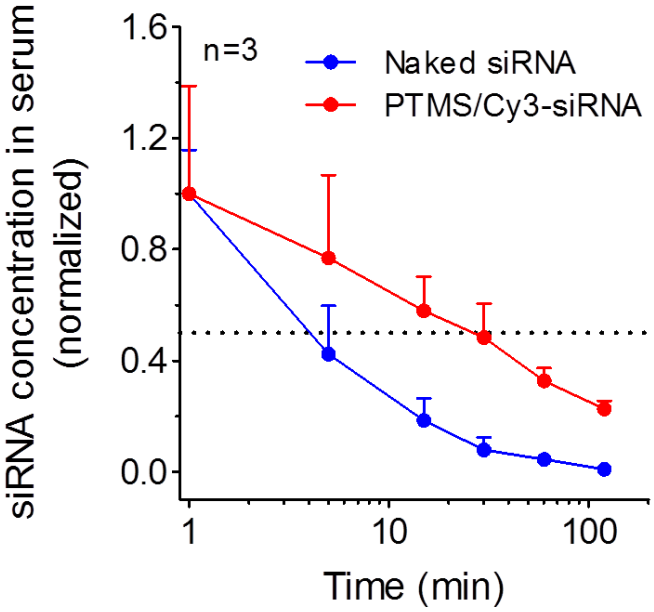

Fig. S8. Pharmacokinetics study of PTMS/Cy3-siRNA formulation in C57BL/6 mice. Mice were i.v. injected with PTMS/Cy3-siRNA (N/P=15) formulation at the dose of 1mg/kg. At given time-points, serum was collected and Cy3-siRNA was analyzed with 550nm excitation and 580nm emission.