

Article

# Targeted PHA Microsphere-Loaded Triple-Drug System with Sustained Drug Release for Synergistic Chemotherapy and Gene Therapy

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## Supplementary Materials:

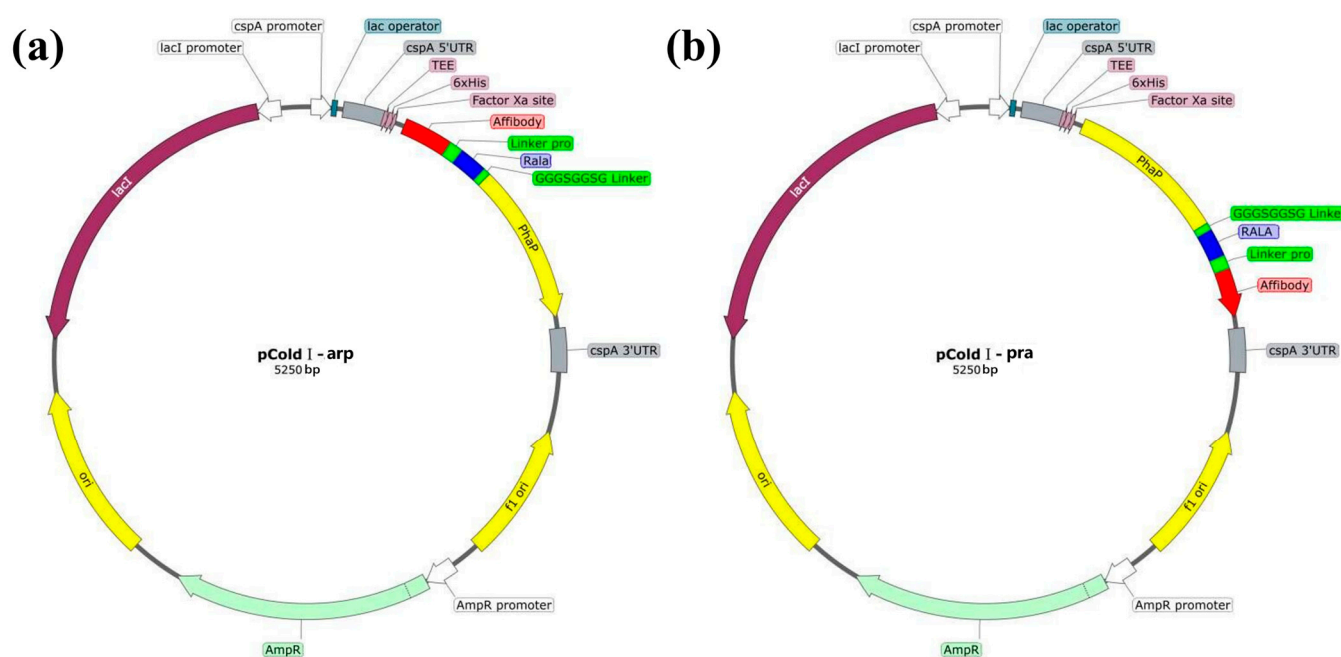


Figure S1. Expression plasmids containing two fusion protein genes of different orders. (a) pCold I-affibody-rala-phaP (pCold I-arp). (b) pCold I-phaP-rala-affibody (pCold I-pra).

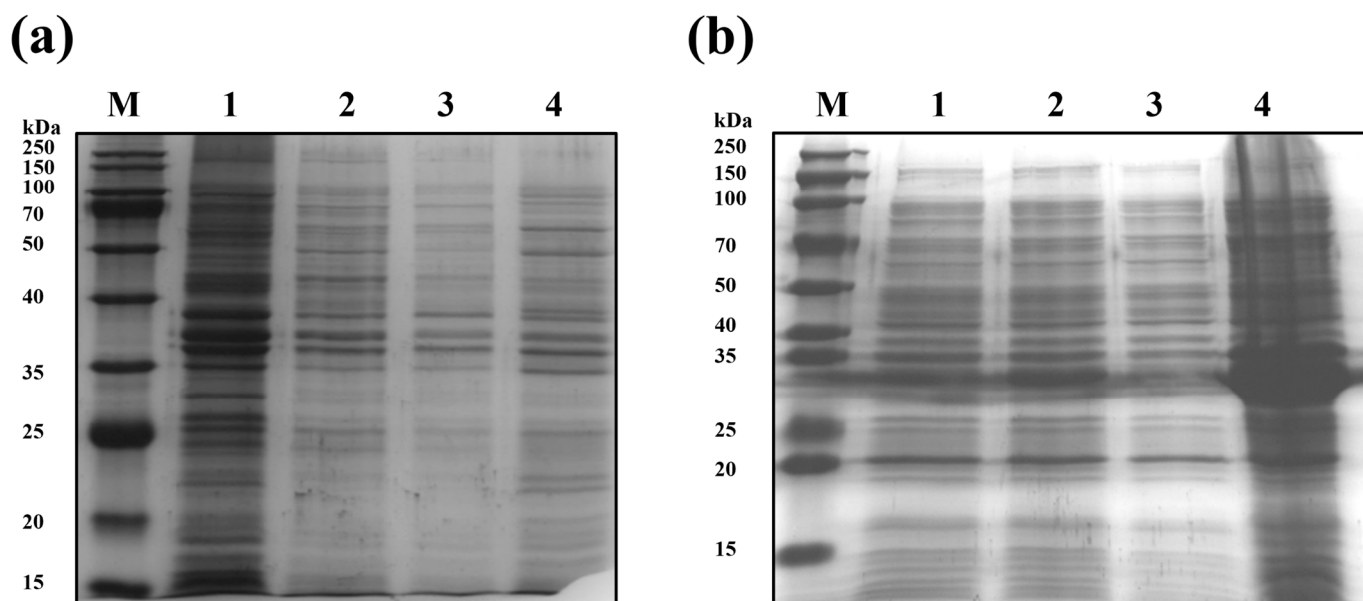


Figure S2. (a) Analysis of PRA protein by SDS-PAGE gel electrophoresis. (M: Marker; Lane 1: before IPTG induction; Lane 2: after IPTG induction; Lane 3: supernatant after crushing; Lane 4: bacterial precipitation after crushing). (b) Analysis of ARP proteins by SDS-PAGE gel electrophoresis. (Lane M: Marker; Lane 1: supernatant of crushed cells; Lane 2: after induction of ARP protein; Lane 3: before induction of ARP protein; Lane 4: bacteria precipitate after fragmentation).

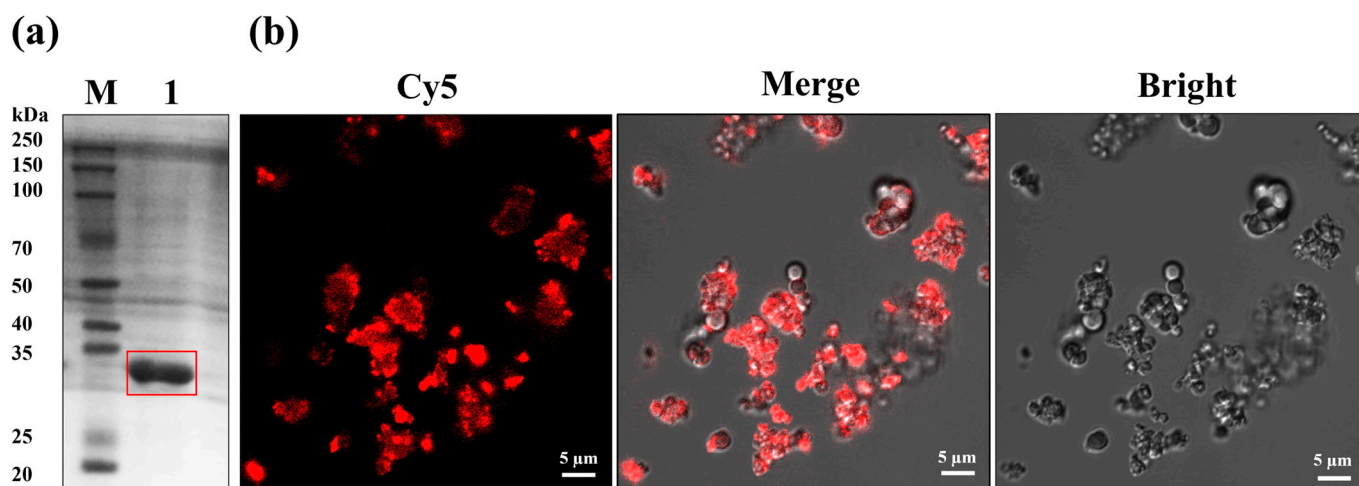


Figure S3. Verification of the correctly assembled triple-drug microspheres. (a) SDS analysis of the ARP protein was attached to the surface of the microspheres. (b) Confocal imaging validation of siRNA<sub>GEM</sub> was absorbed by microspheres.

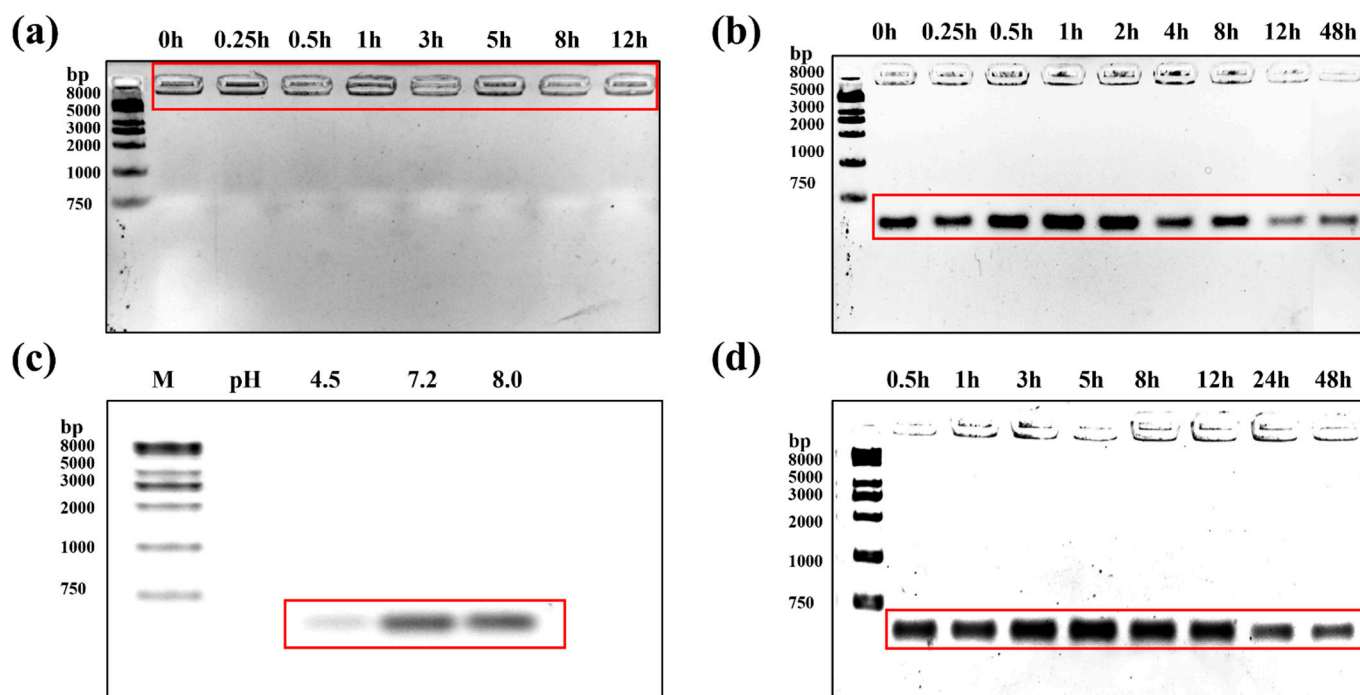


Figure S4. Analysis of the enzymatic release of PTX@PHBHHx-ARP/siRNA<sub>GEM</sub> by agarose gel electrophoresis. (a) The stability of PTX@PHBHHx-ARP/siRNA<sub>GEM</sub> at different times in 10% serum without heparin sodium treatment. (b) The stability of siRNA<sub>GEM</sub> on PTX@PHBHHx-ARP/siRNA<sub>GEM</sub> at different times in 10% serum with heparin sodium treatment. (c) The stability of PTX@PHBHHx-ARP/siRNA<sub>GEM</sub> at different pH. (d) The stability of PTX@PHBHHx-ARP/siRNA<sub>GEM</sub> in RNase I.

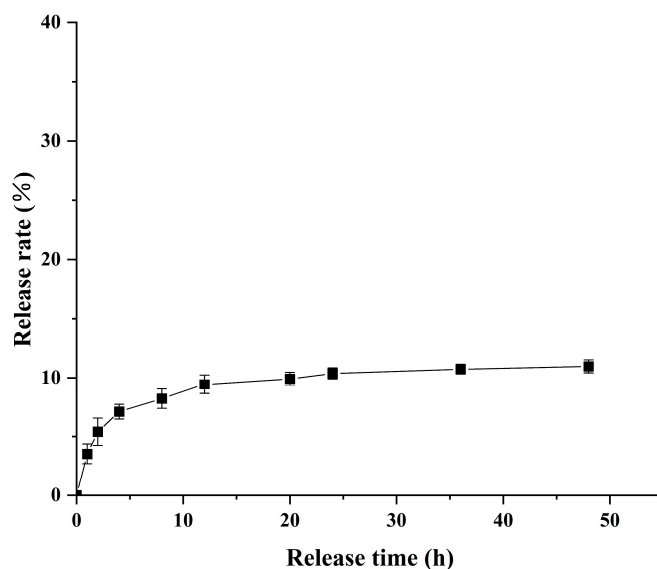


Figure S5. Kinetics of PTX release under neutral conditions.

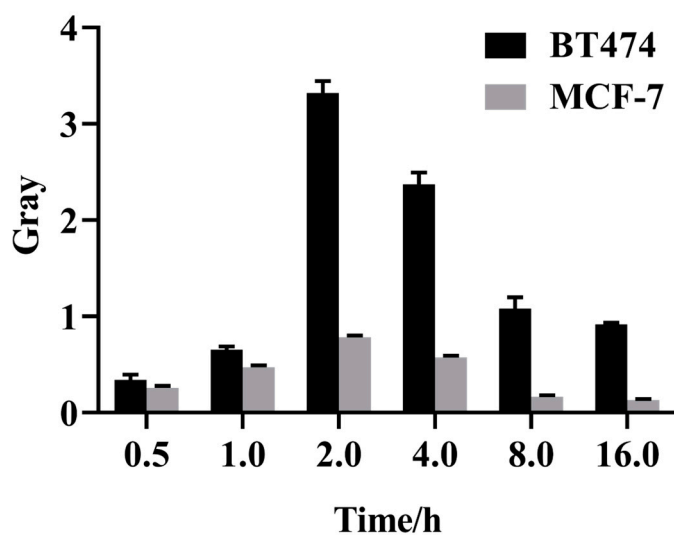


Figure S6. Grayscale control of uptake of PTX@PHBHx-ARP/siRNA<sub>GEM</sub> by two different cells.

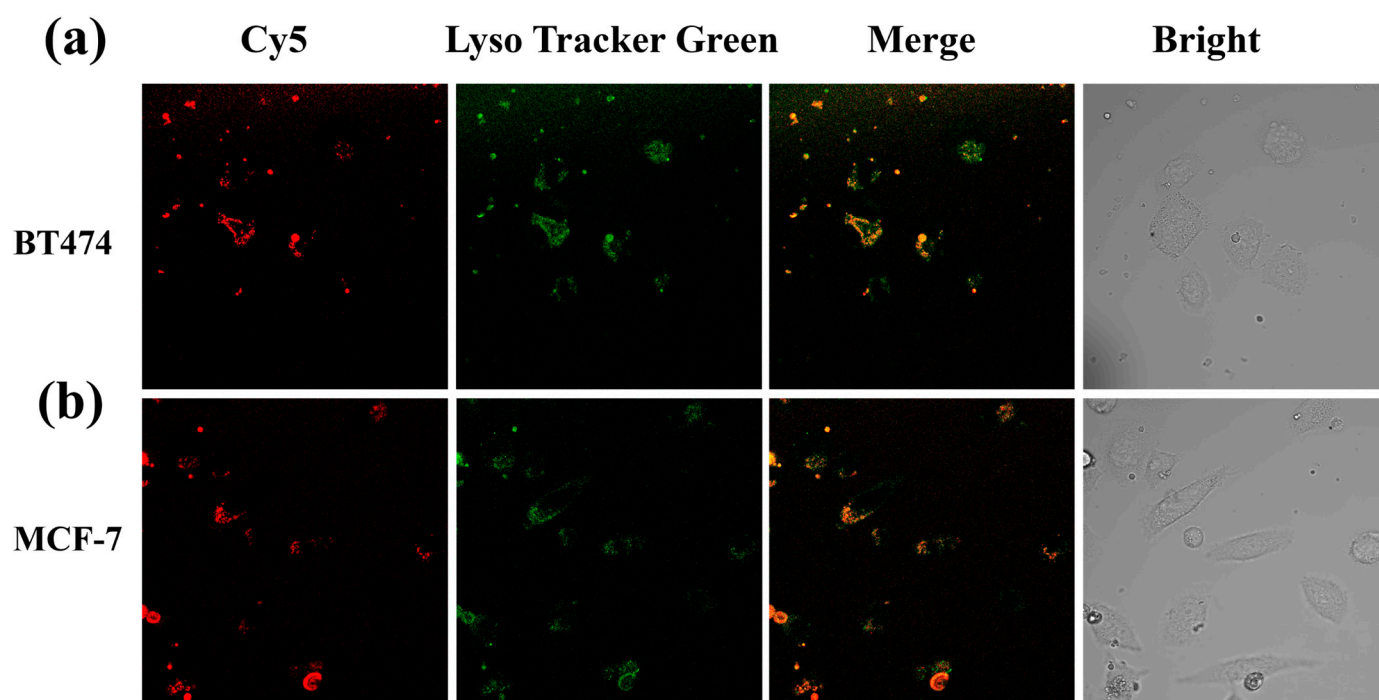


Figure S7. Intracellular localization map of the drug after uptake by BT474 (a) and MCF-7 (b) cells. After the cells were incubated with the drug for 2 h, the position of PTX@PHBHx-ARP/siRNA<sub>GEM</sub> after its entry into the cells was observed by CLSM. Scale bar: 20 μm.

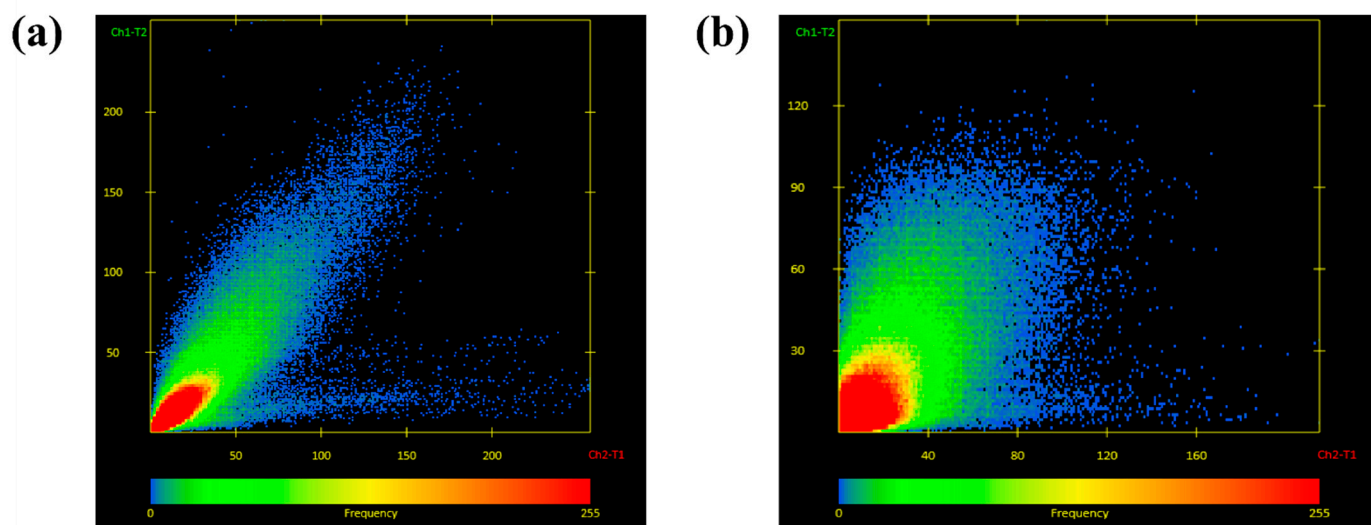


Figure S8. The co-localization of PTX@PHBHx-ARP/siRNA<sub>GEM</sub> in lysosomes was analyzed by software. (a) The co-localization of PTX@PHBHx-ARP/siRNA<sub>GEM</sub> in MCF-7 cells. (b) The co-localization of PTX@PHBHx-ARP/siRNA<sub>GEM</sub> in BT474 cells.

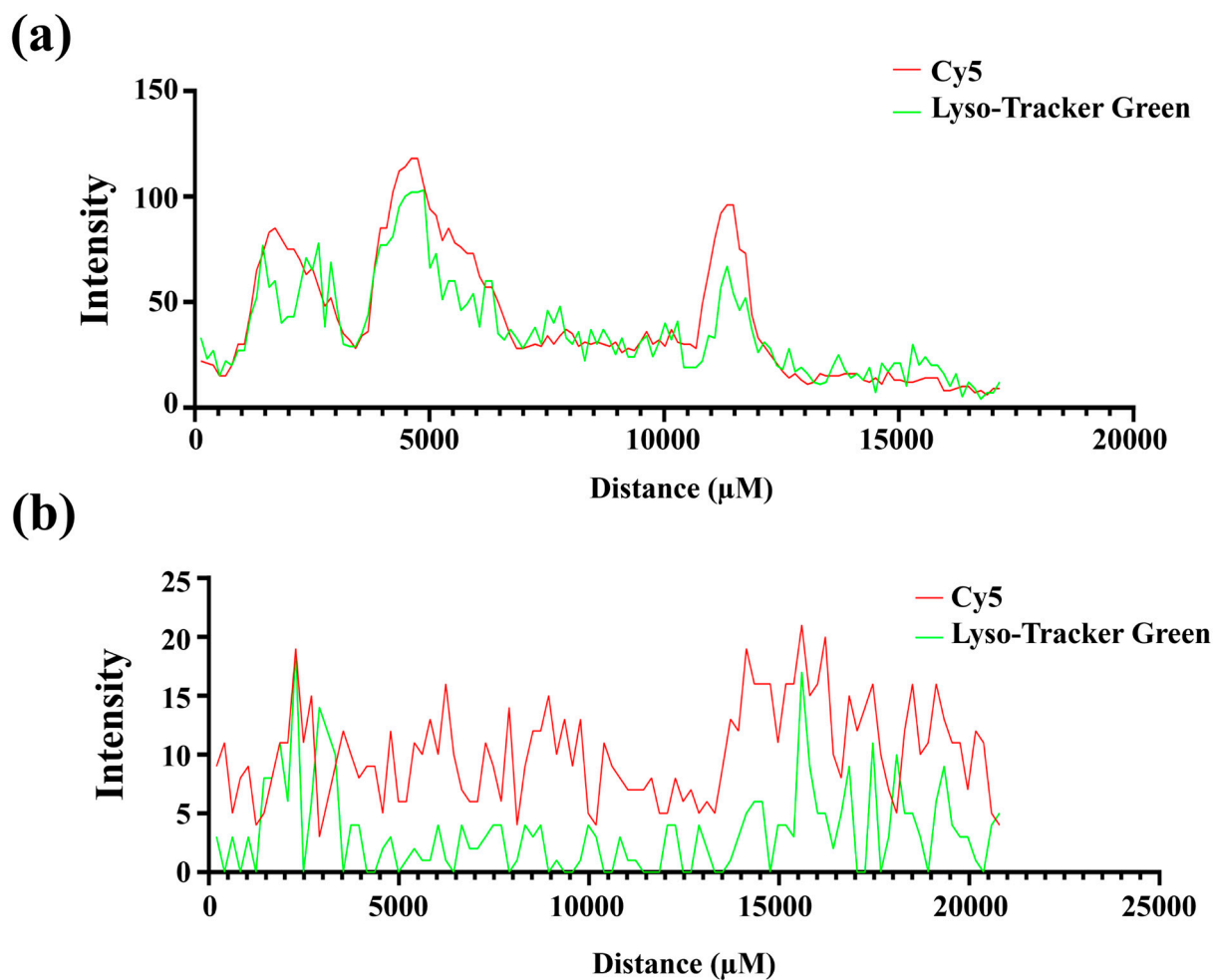


Figure S9. Localization analysis of PTX@PHBHx-ARP/siRNA<sub>GEM</sub> in lysosomes in both cell types. (a) Lysosomal co-localization in BT474 cells. (b) Lysosomal co-localization in MCF-7 cells.

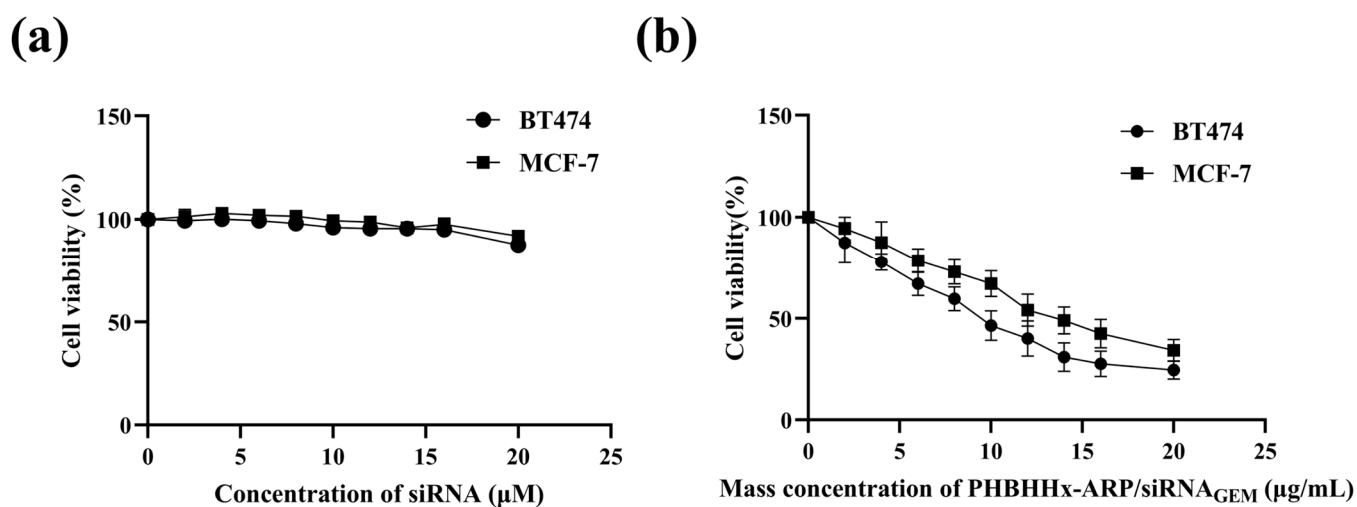


Figure S10. The survival rates of the two cell lines were observed after 48 h of treatment. (a) siRNA. (b) PHBHHx-ARP/siRNA<sub>GEM</sub>.

Table S1

CI values of different cells treated with three different combinations of drugs

Cell line	CI	
	PTX/GEM/siRNA(1:9:170)	PTX@PHBHHx-ARP/siRNA <sub>GEM</sub>
BT474	0.059	0.0007
MCF-7	0.150	0.0014