Electronic Supplementary Information

Enhanced Protein Internalization and Efficient Endosomal Escape Using Polyampholyte-Modified Liposomes and Freeze Concentration

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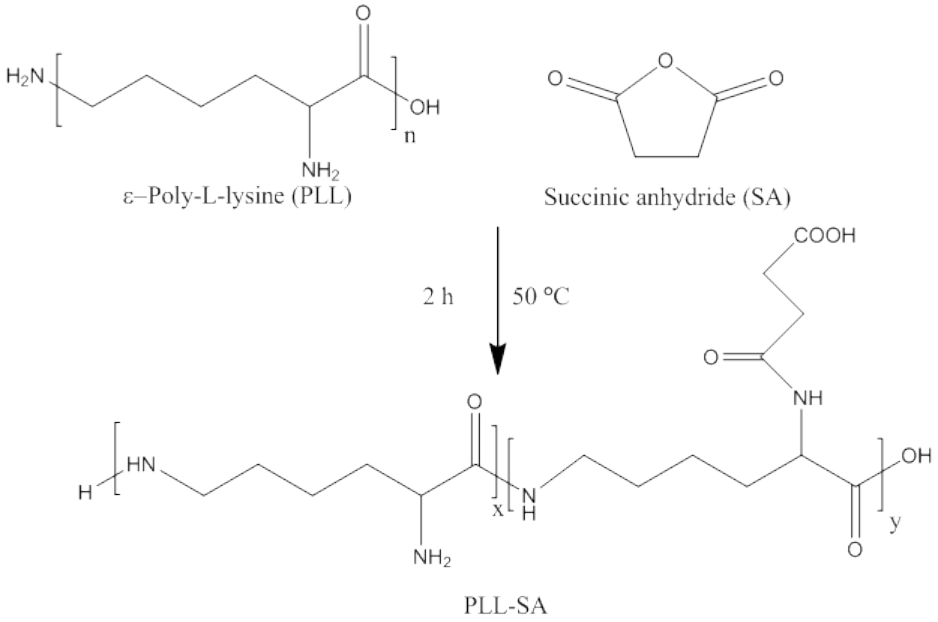
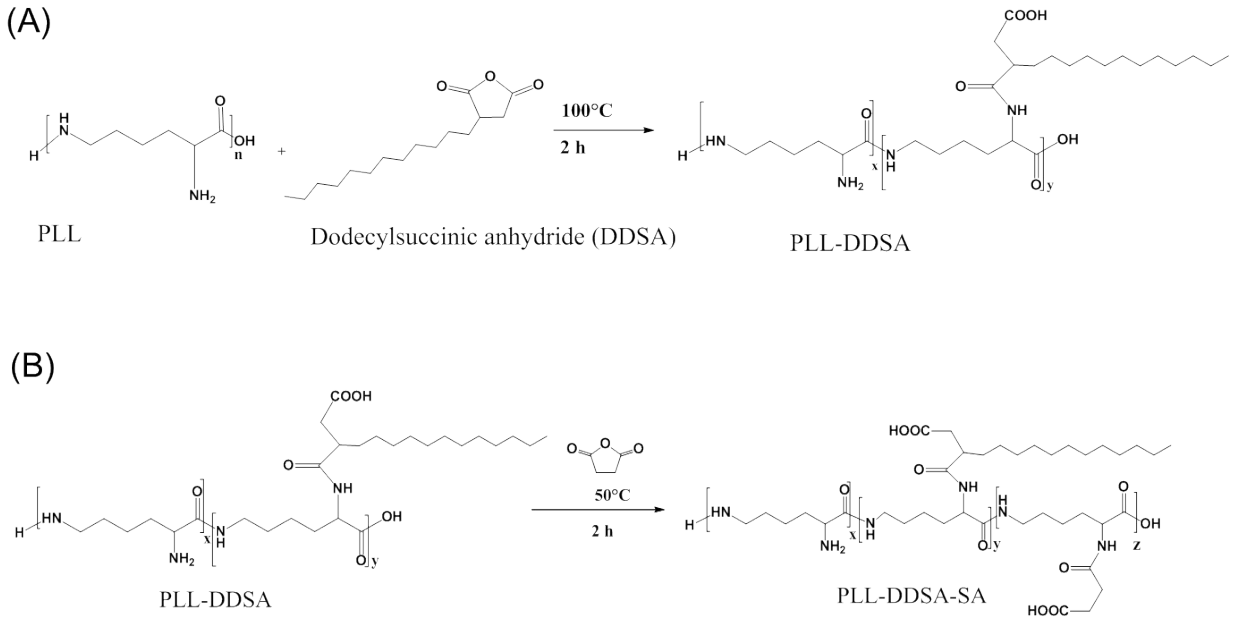


Scheme S1. Generation of the polyampholyte cryoprotectant. PLL-SA was prepared using succinic anhydride.



Scheme S2. Preparation of hydrophobically modified polyampholytes (PLL-DDSA-SA). (A) DDSA reaction with PLL. (B) SA reaction with DDSA-PLL

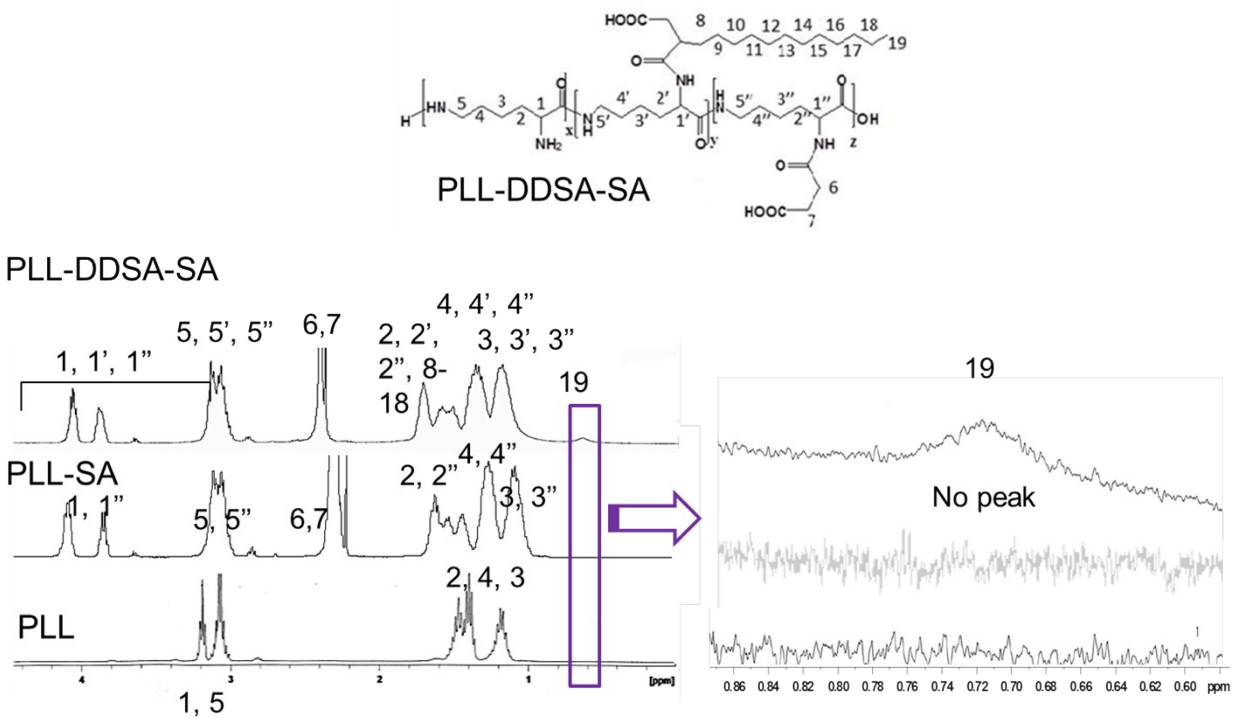


Figure S1. ^{1}H-NMR spectra of hydrophobically modified polyampholyte (PLL-DDSA-SA), polyampholyte (PLL-SA), and unmodified PLL.

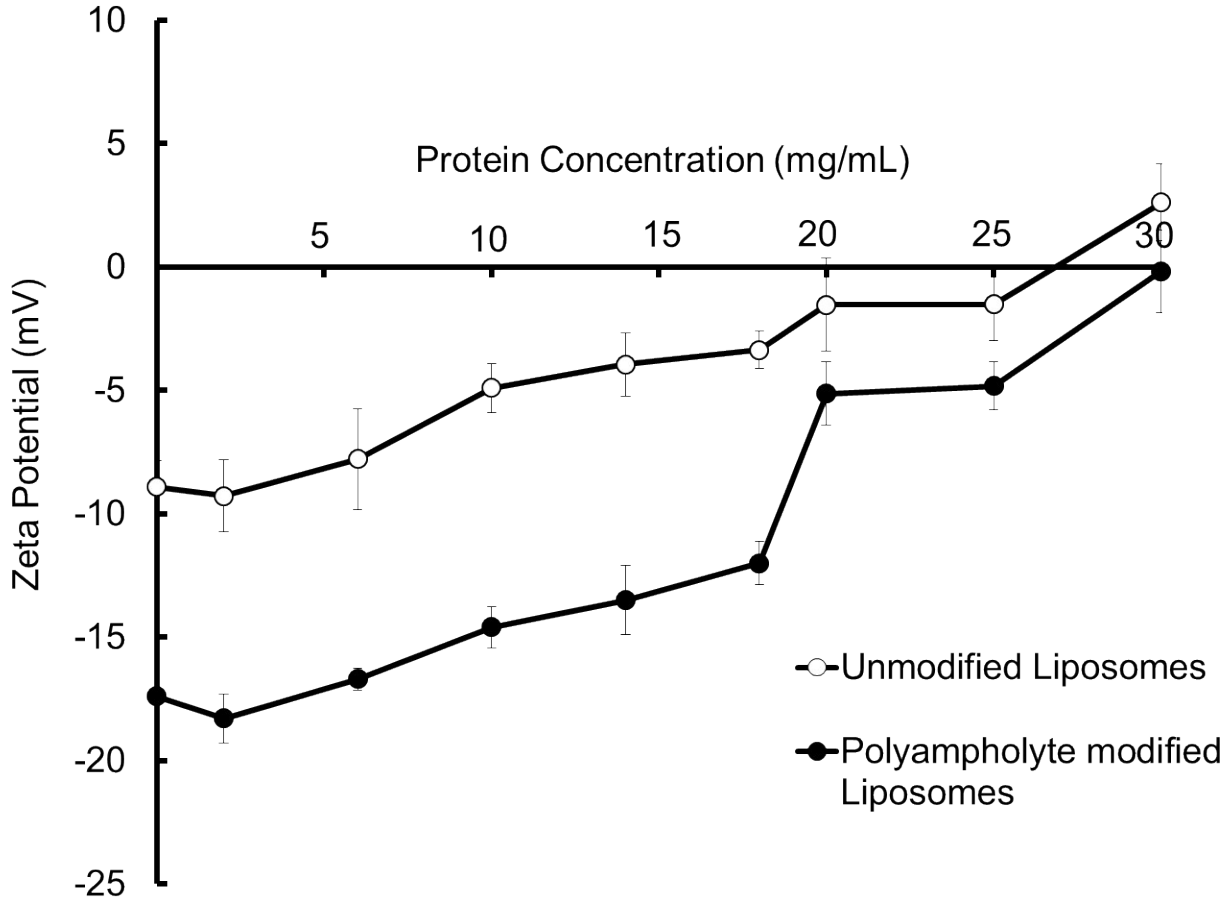


Figure S2. Zeta potentials of different concentrations of encapsulated proteins on unmodified liposomes and polyampholyte-modified liposomes.

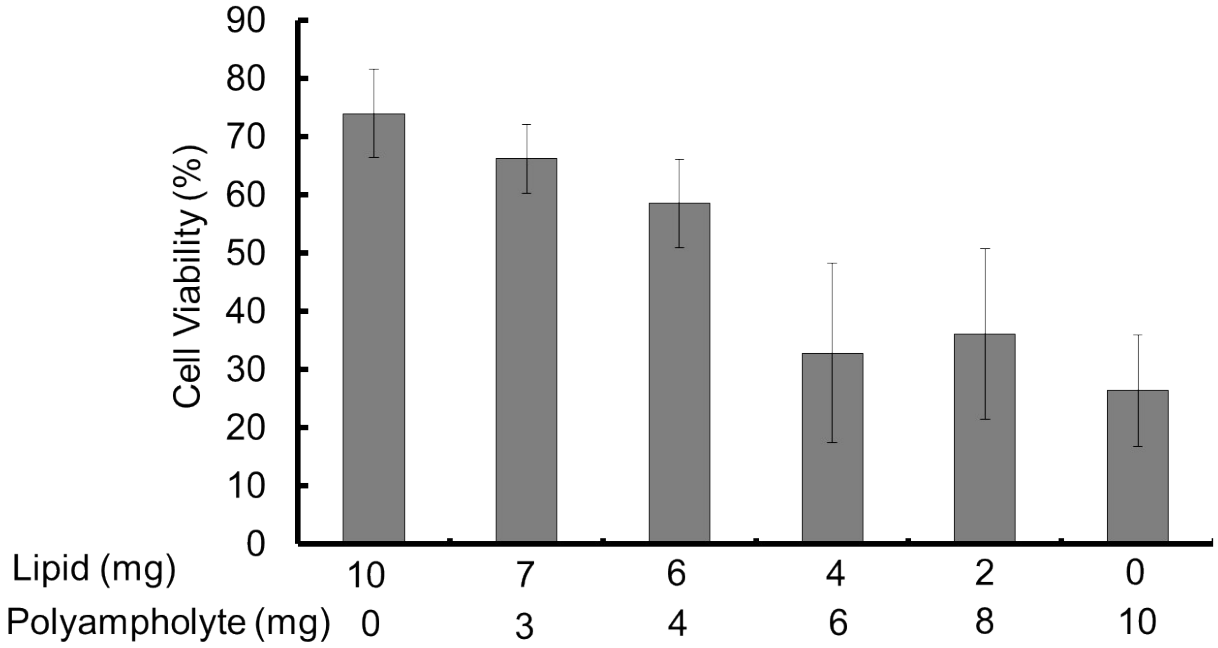


Figure S3. Cytotoxicity of polyampholyte-modified liposomes with encapsulated lysozyme (10 mg/mL). L929 cells were incubated for 48 h with different ratios of liposomes and polyampholytes and a constant amount of lysozyme protein (10 mg/mL), followed by MTT assay analysis. Data are expressed as the mean ± standard deviation (SD).

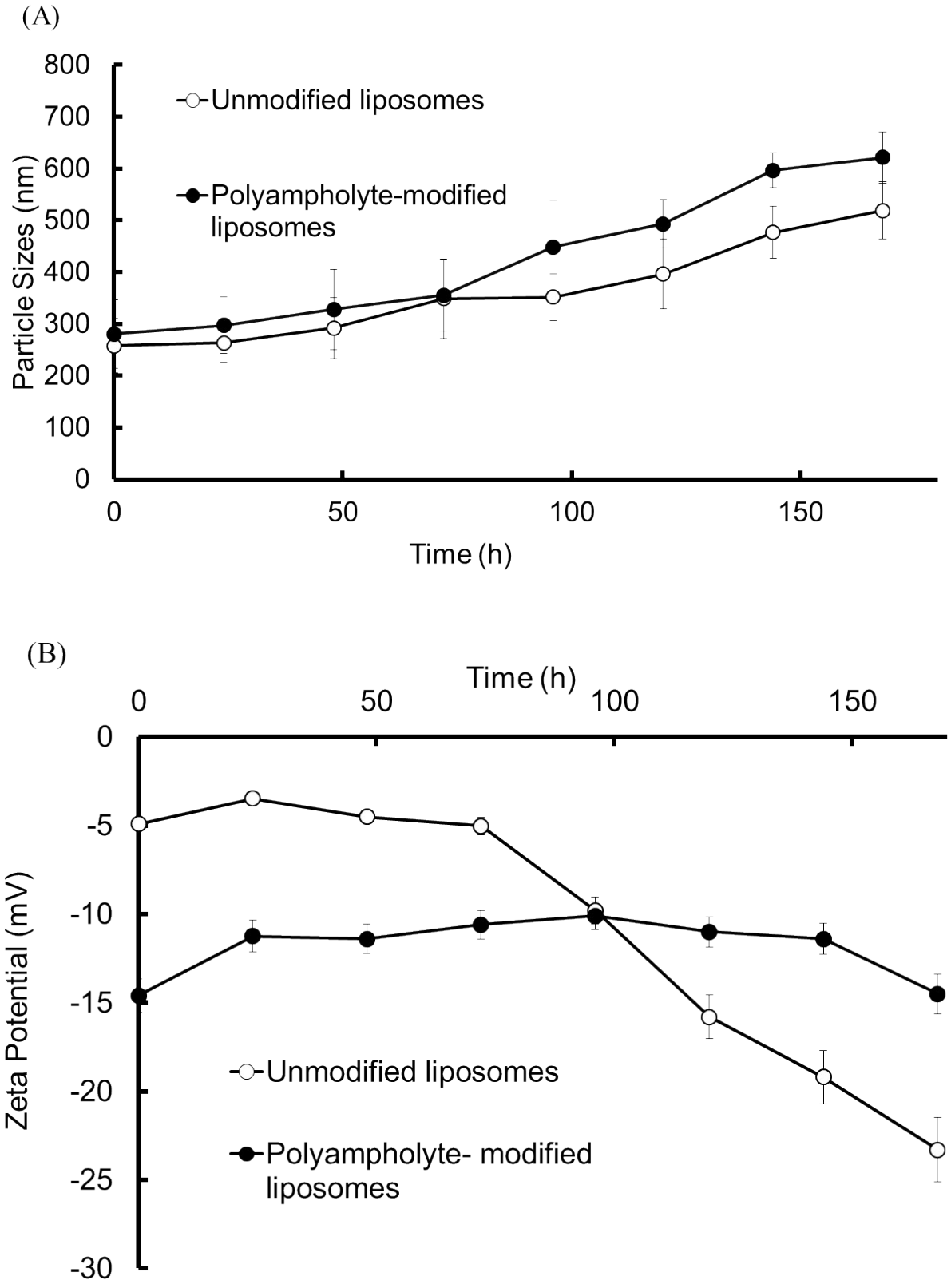


Figure S4. Unmodified and polyampholyte-modified liposome-encapsulated proteins were incubated for 7 days at 25 °C. (A) Particle size; (B) Zeta potential.

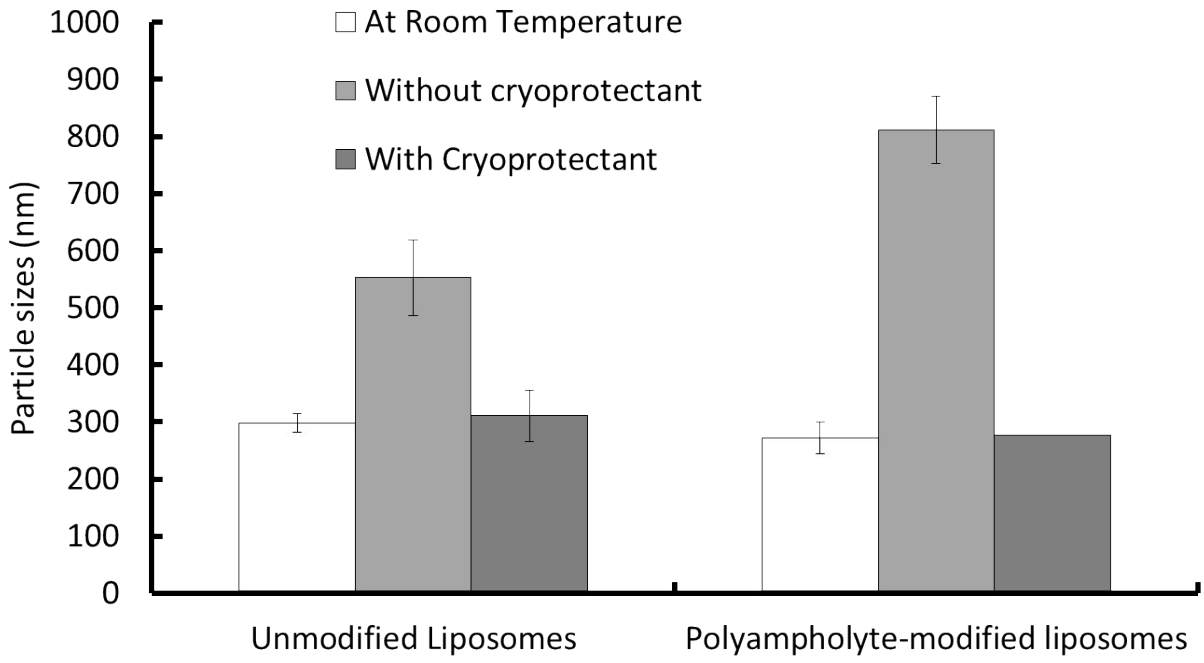


Figure S5. Particle sizes of unmodified liposomes and polyampholyte-modified liposomes were determined by DLS at 25ºC and at −80ºC, with and without 10% PLL-SA cryoprotectant. Data are expressed as the mean ± SD.

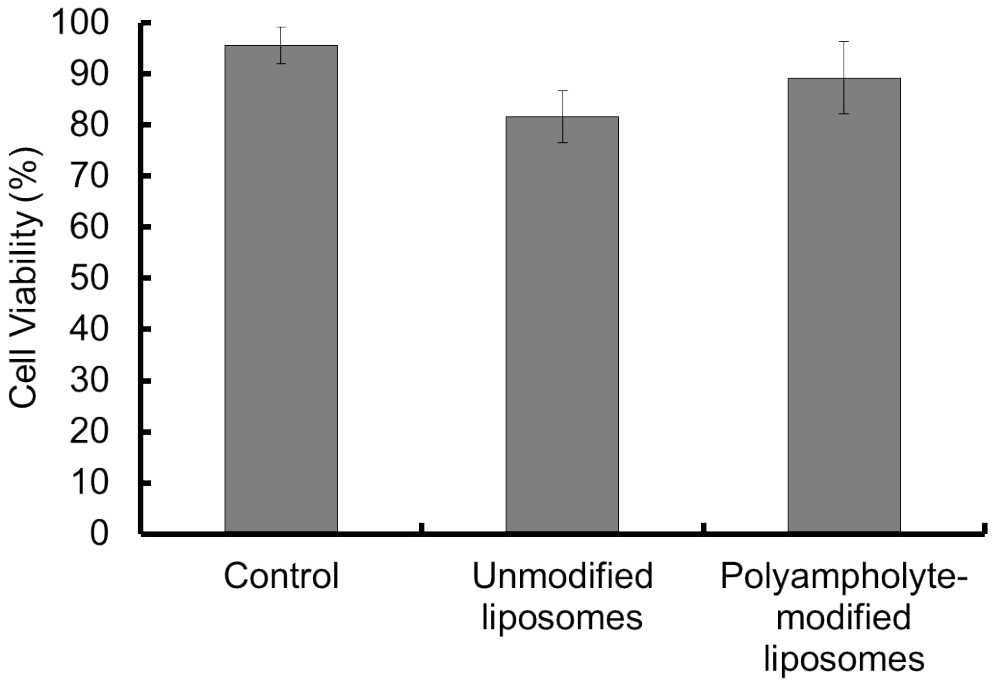


Figure S6. Cell viability after storage at −80 °C for 1 day along with unmodified or polyampholyte-modified liposomes in the presence of the polymeric cryoprotectant PLL-SA. Data are expressed as the mean ± SD.

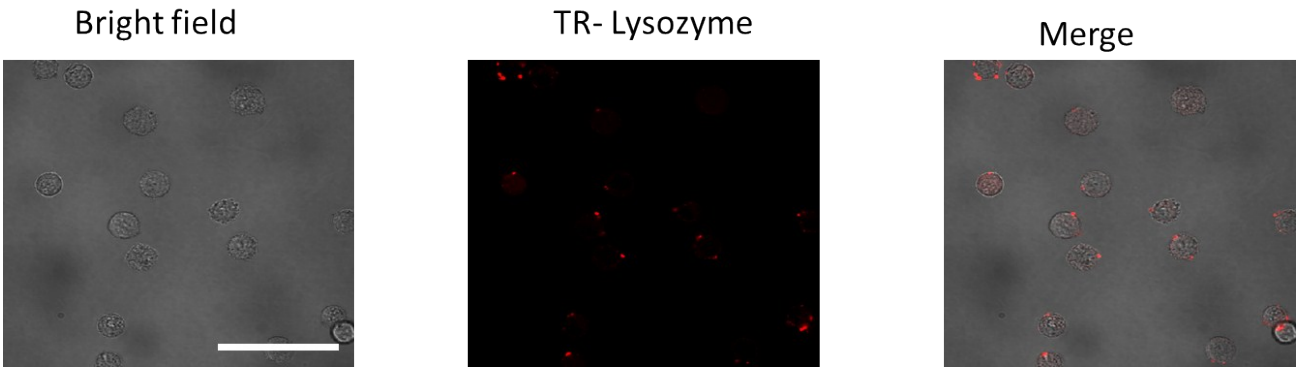


Figure S7. Confocal microphotograph of L929 cells (without freezing), using bare lysozyme proteins. Lysozyme proteins were stained with TR red. Scale bar: 10 μm

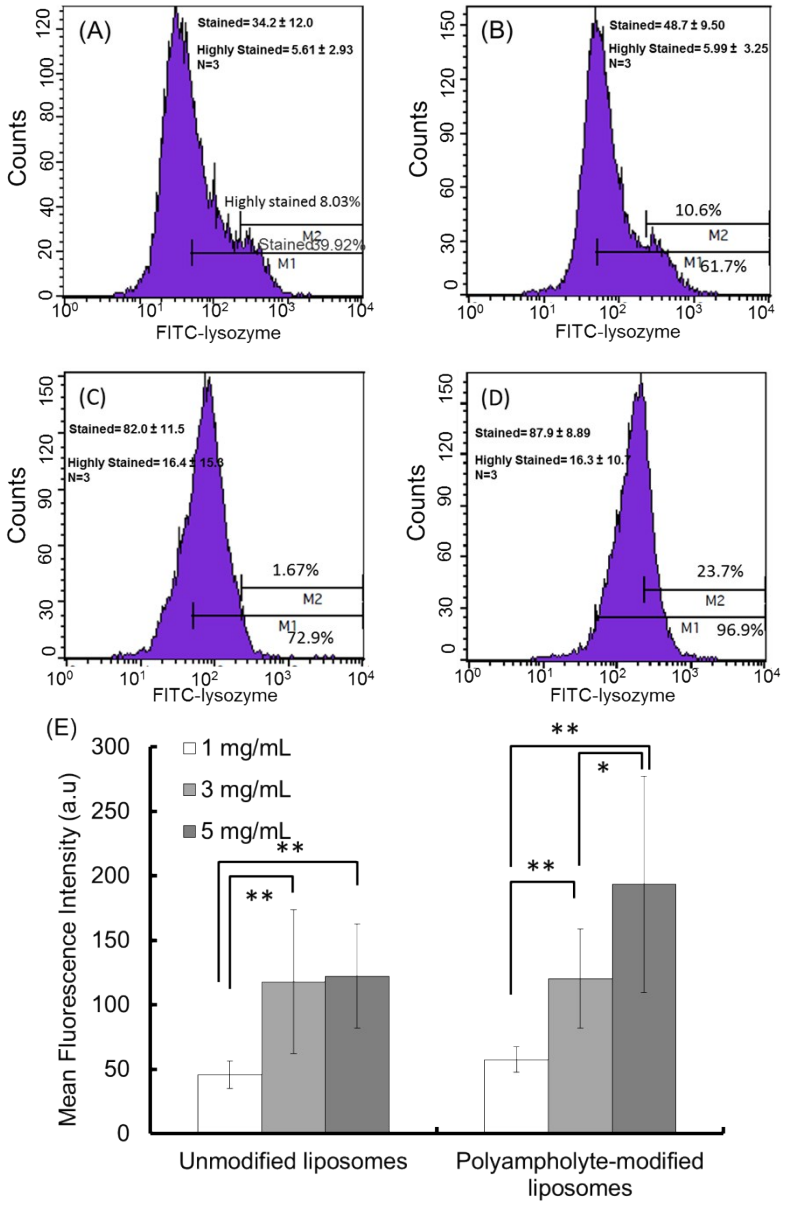
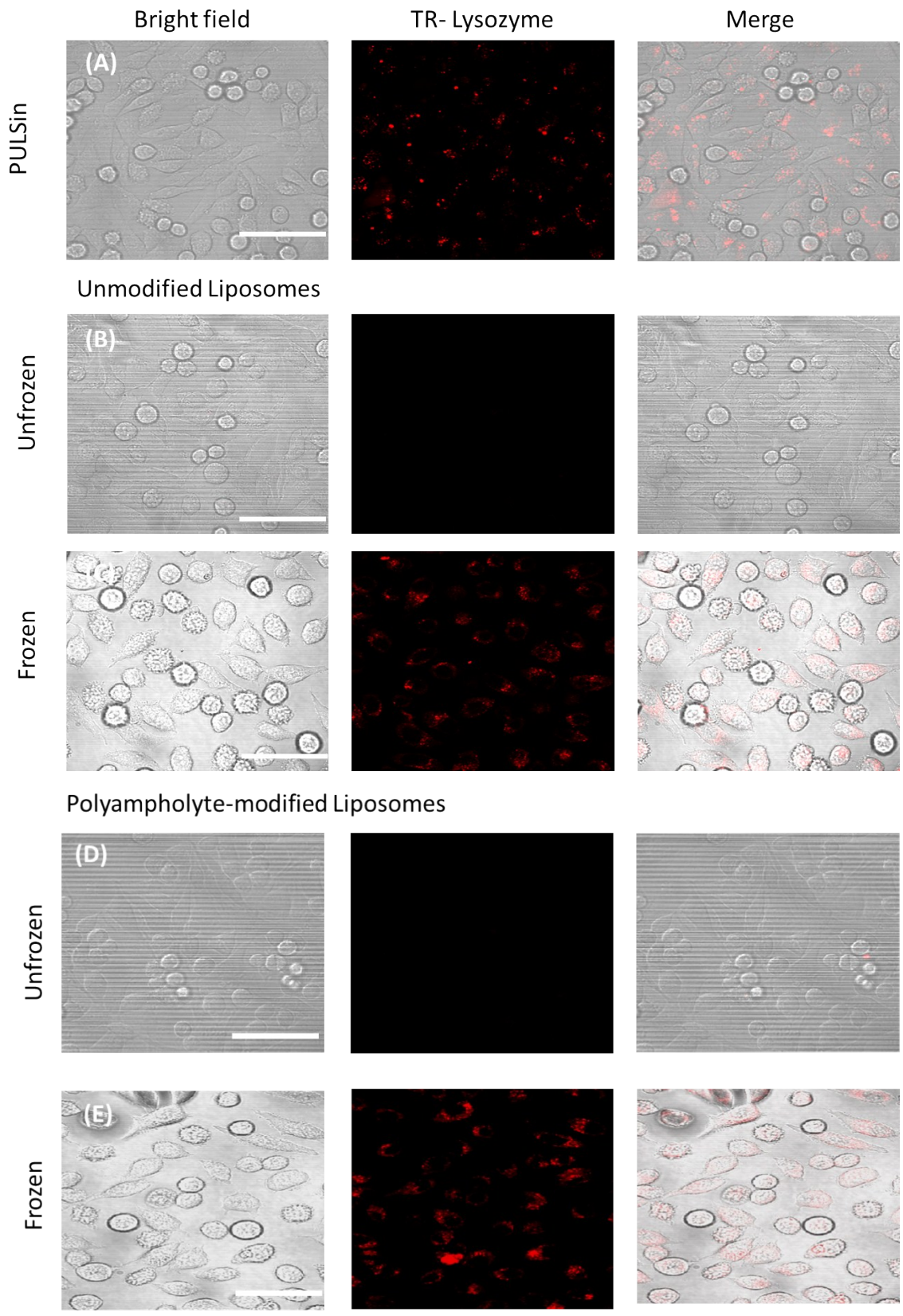


Figure S8. Flow cytometric quantification of the fluorescence intensity of cells before and after being frozen with various protein-loaded liposomes. We used 1×10^6 cells for sample preparation and analysis by flow cytometry, under the following conditions. (A) Low dose (1 mg/mL) of unmodified liposomes. (B) low dose (1 mg/mL) of polyampholyte-modified liposomes. (C) Medium dose (3 mg/mL) of unmodified liposomes. (D) Medium dose (3 mg/mL) of polyampholyte-modified liposomes. (E) Mean fluorescent intensity showing the dose dependency of FITC-conjugated lysozyme loading using unmodified and polyampholyte-modified liposomes. Data are expressed as the mean ± SD. **P < 0.01, *P < 0.05


Bright field
TR- Lysozyme
Merge
PULSin
(A)
Unmodified Liposomes
Unfrozen
(B)
Frozen
Polyampholyte-modified Liposomes
Unfrozen
(D)
Frozen
(E)

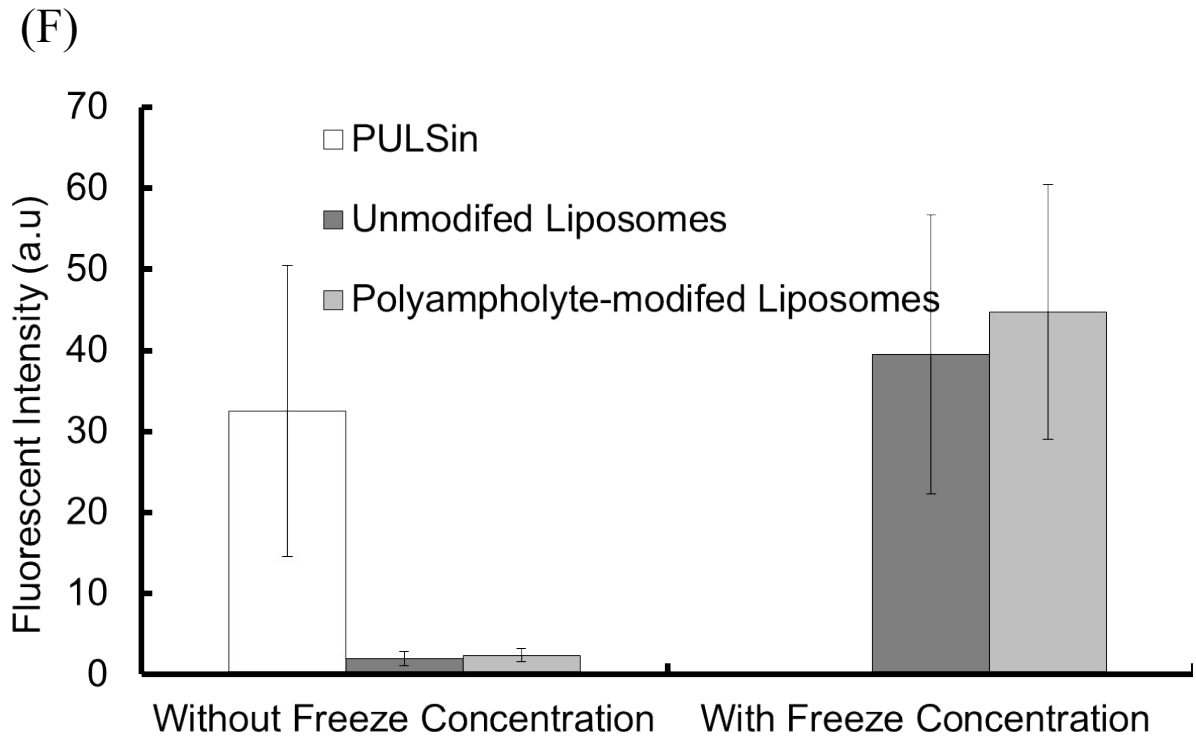


Figure S9. Confocal microphotograph showing internalization of TR-labelled lysozyme proteins in L929 cells. Cells were analysed after a 6-h incubation, which was followed by adding lysozyme protein/carrier complexes. (A) PULSin/lysozyme protein complex (B, D) without freeze concentration of unmodified and polyampholyte-modified liposome/protein complexes. (C,E) Images taken after freeze concentration of unmodified and polyampholyte-modified liposomes. (F) Mean fluorescent intensity following internalization of the positive control (PULSin), or unmodified and polyampholyte modified liposomes, with or without freeze concentration. Data are expressed as the mean ± SD.

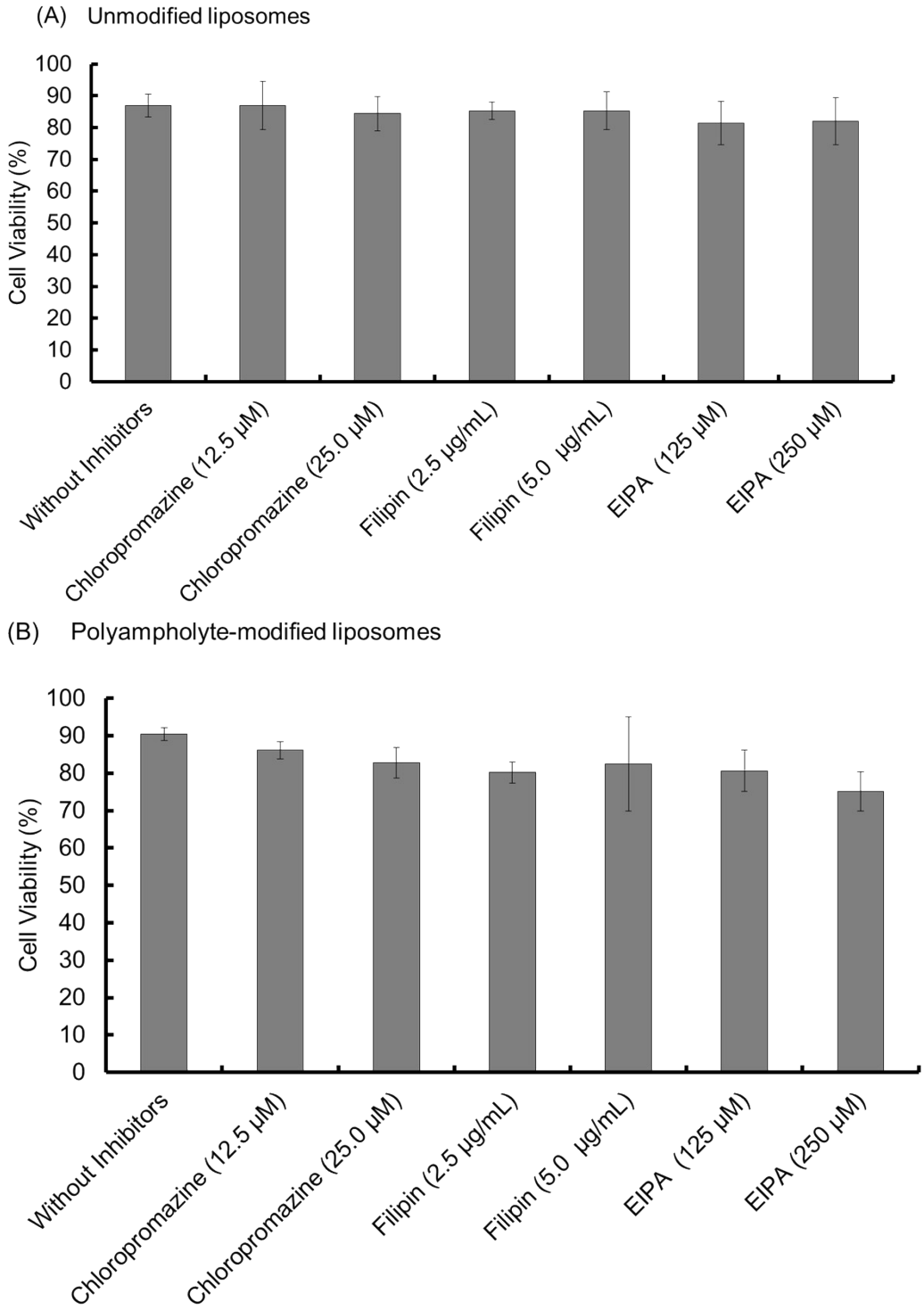


Figure S10. Quantification of cell viability determined by trypan blue-exclusion assays after the addition of different concentrations of endocytic inhibitors to protein-liposome complexes. (A) Unmodified liposomes. (B) Polyampholyte-modified liposomes. Data are expressed as the mean ± SD.