Supporting Information

PEG-coated nanoparticles detachable in acidic microenvironments for the tumor-directed delivery of chemo- and gene therapies for head and neck cancer

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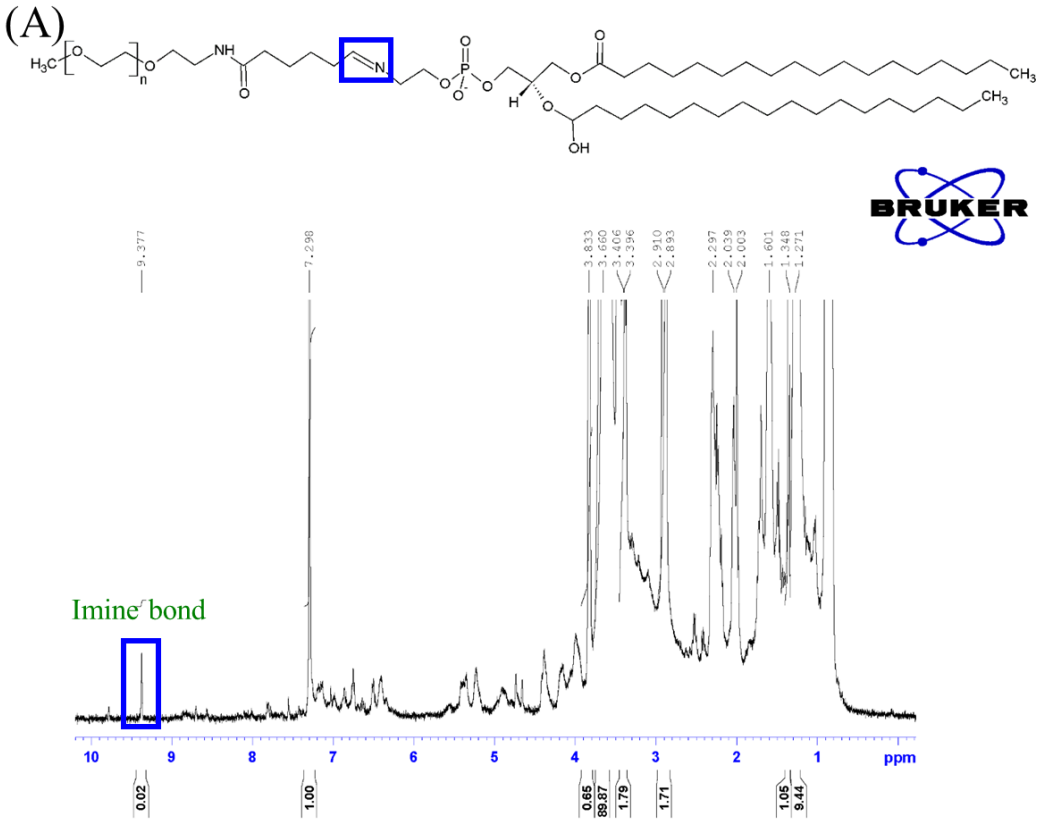
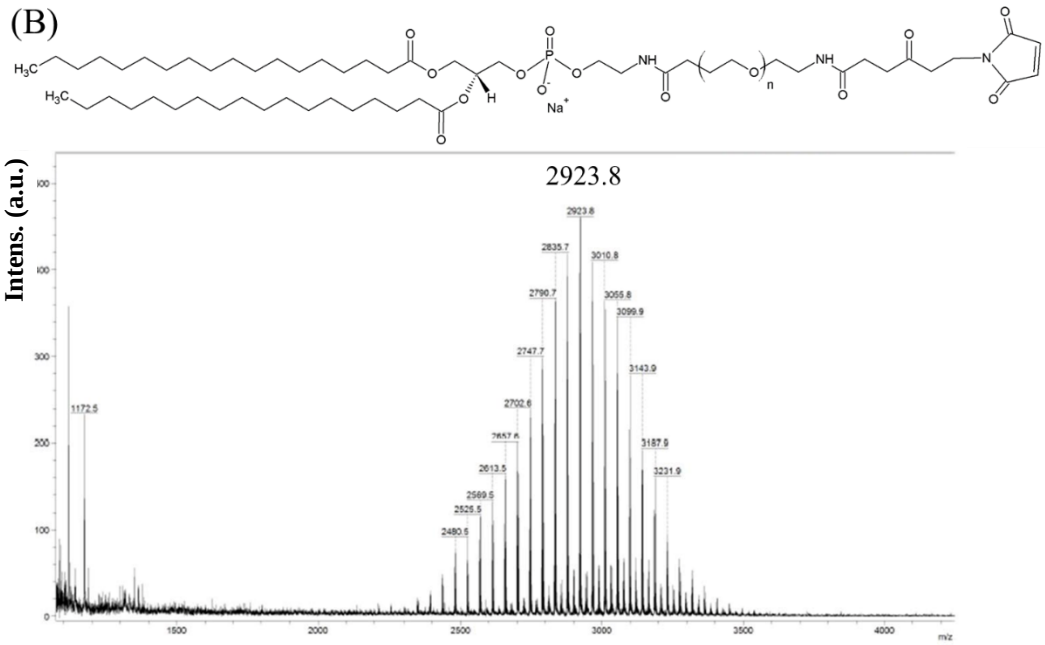
Supplementary Figures





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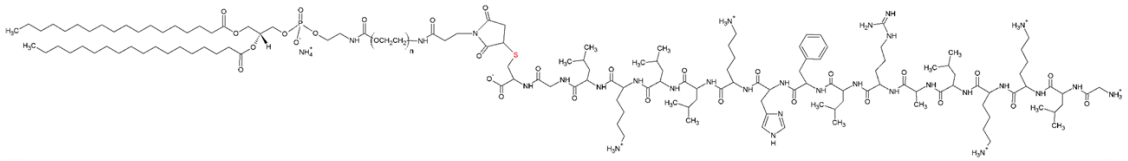

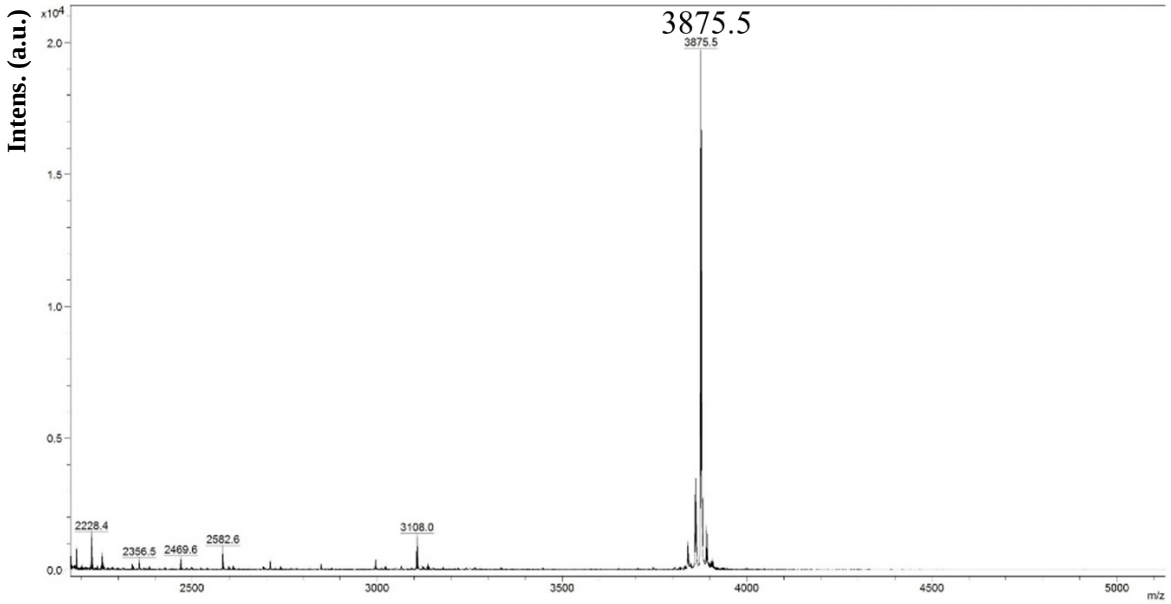
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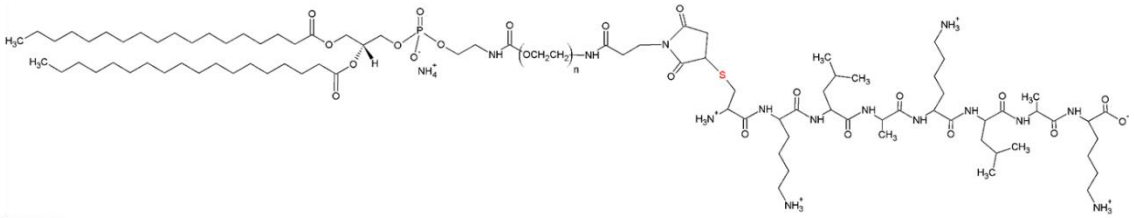

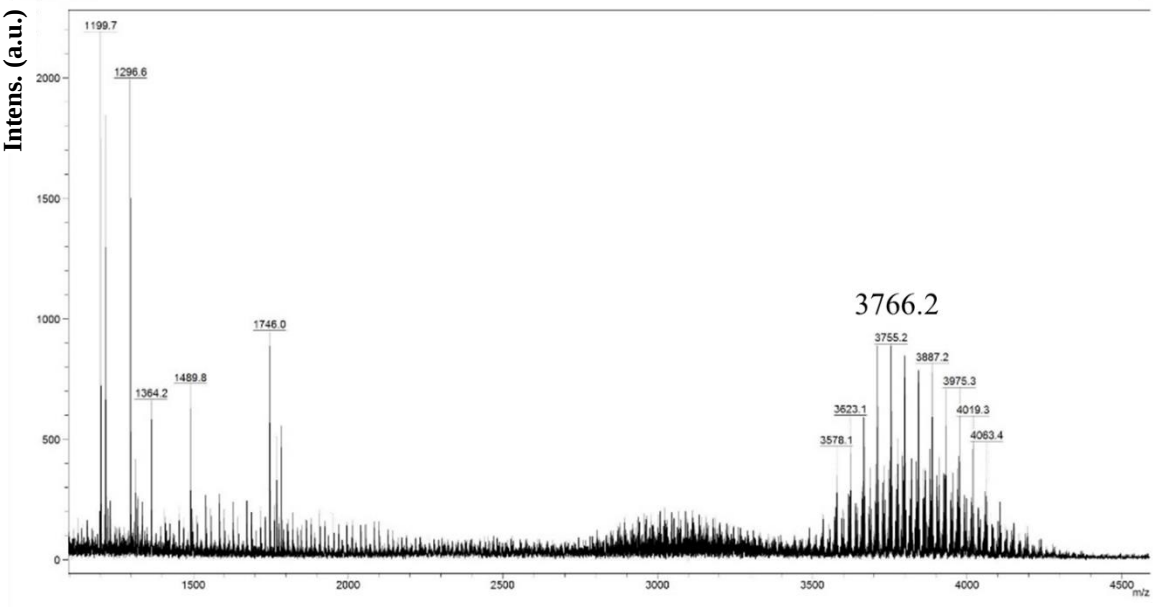
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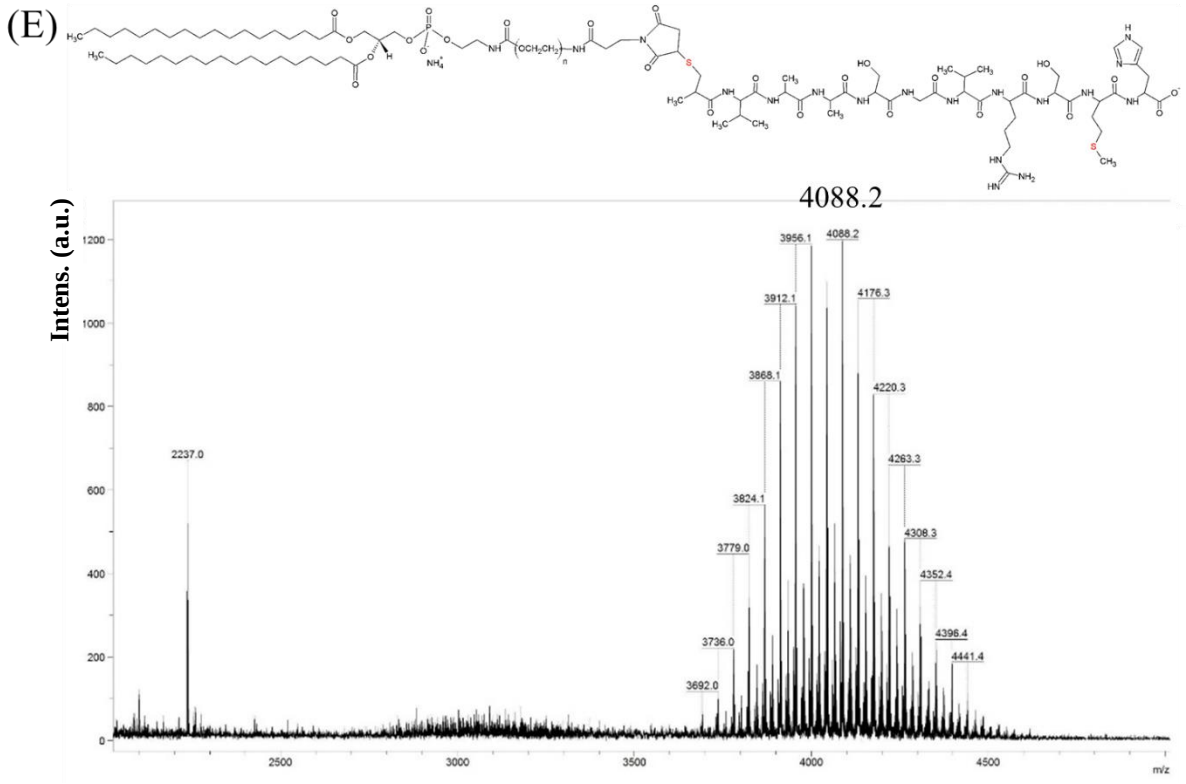

Figure S1. NMR and mass spectrometric characterization of DSPE-omPEG and peptide-modified DSPE-PEG. (A) DSPE was conjugated to omPEG in organic solvent for 24h and the product DSPE-omPEG was detected by ^{1}H NMR. (B-E) Different peptides were conjugated to DSPE-PEG-maleimide. The mass spectra of (B) DSPE-PEG-maleimide and the products of (C) DSPE-PEG-N, (D) DSPE-PEG-M, and (E) DSPE-PEG-C were detected by MALDI-TOF.

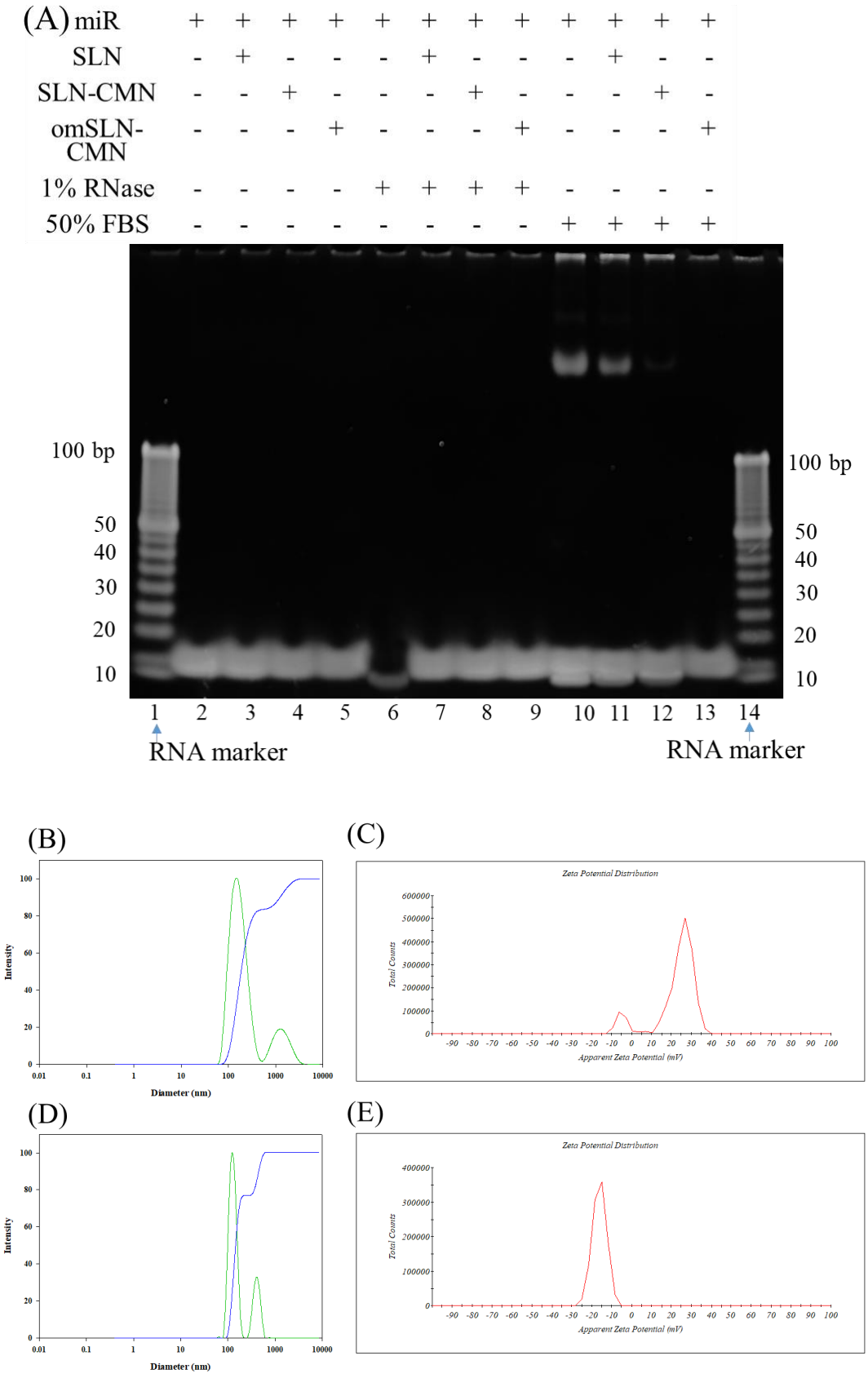

Figure S2. Protection test of miR-loaded formulations by gel retardation assay and characterization of miR-200/omSLN-CMN and Iri/omLip-CMN at pH 6.5. (A) miR was encapsulated with or without different SLN formulations (SLN, SLN-CMN, and omSLN-CMN) and incubated with 1%

RNase or 50% FBS at 37°C for 24 h. The samples were loaded into the gel and run by 8% polyacrylamide at 60 V. After staining with 0.001% ethidium bromide for 30 min at 25°C, the gel was then monitored and scanned using a gel documentation system (DigiGel; TopBio, Taipei, Taiwan). (B-E) Sizes and zeta potential of (B-C) miR/omSLN-CMN and (D-E) Iri/omLip-CMN were measured using a Zetasizer at pH 6.5.

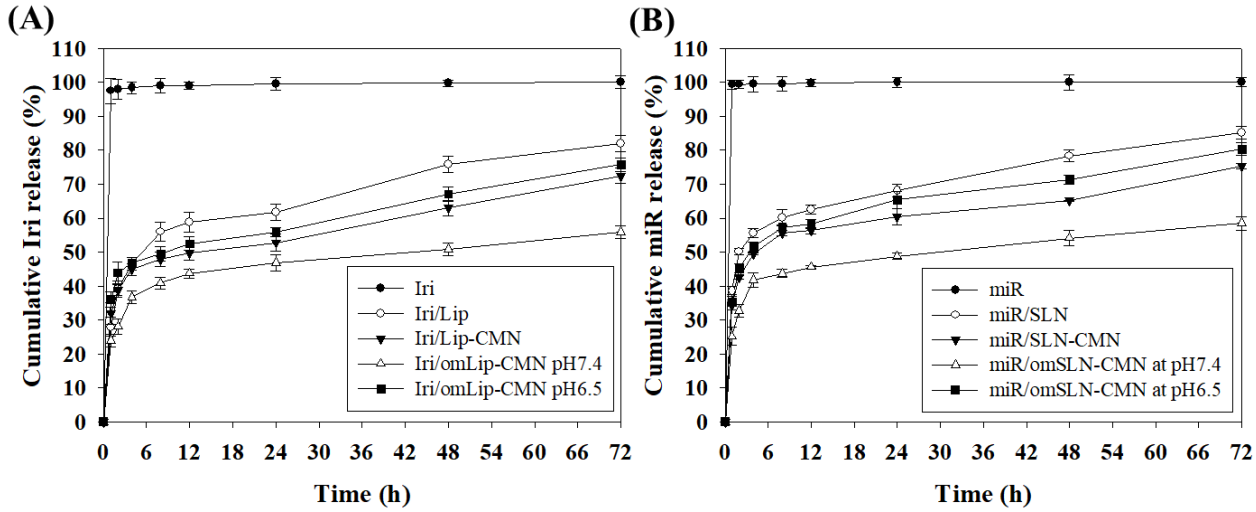

Figure S3. Release profiles of various formulations of (A) irinotecan and (B) miR at 37˚C.

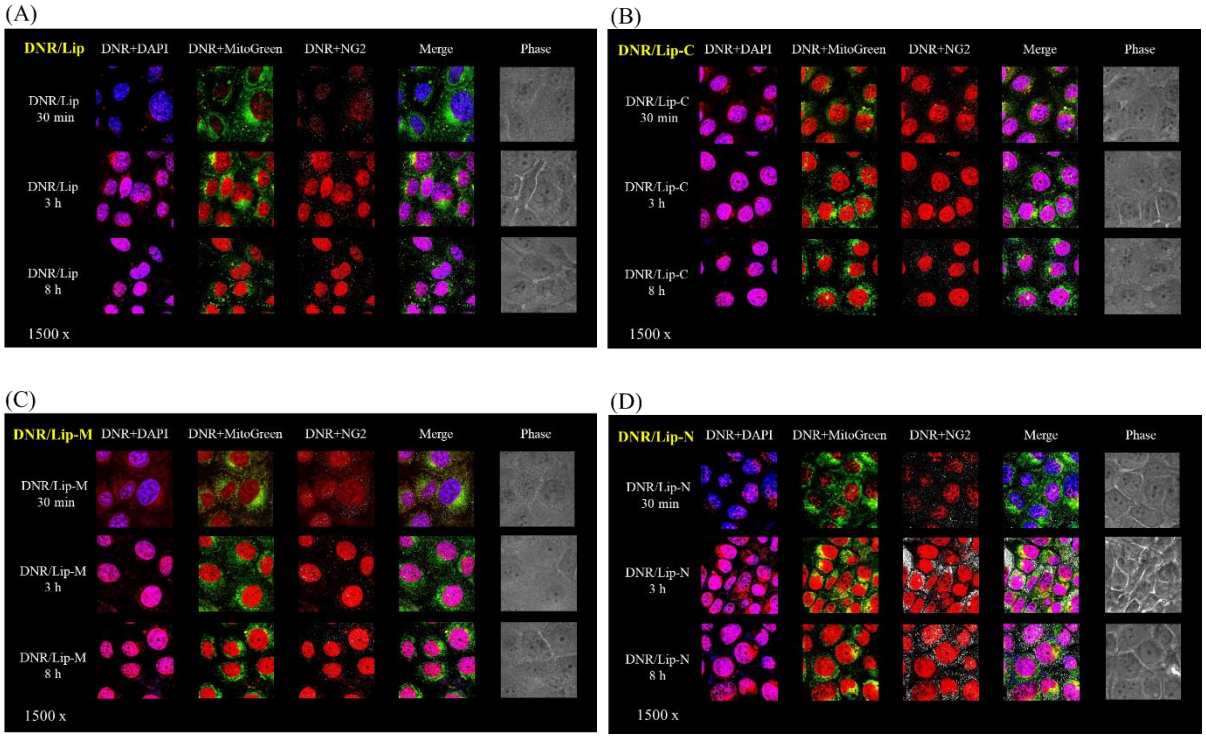

Fig. S4. (A) DNR/Lip, (B) DNR/Lip-C, (C) DNR/Lip-M, and (D) DNR/Lip-N were added to the cells for 30 min, 3 h, and 8 h. Intracellular localization of individual DNR/Lip formulations in SAS cells was observed by CLSM. Blue: DAPI (a nuclear dye); Green: MitoGreen (MitoTracker Green; a mitochondrial dye); Red: DNR; Gray: NG2 (nerve/glial antigen 2).

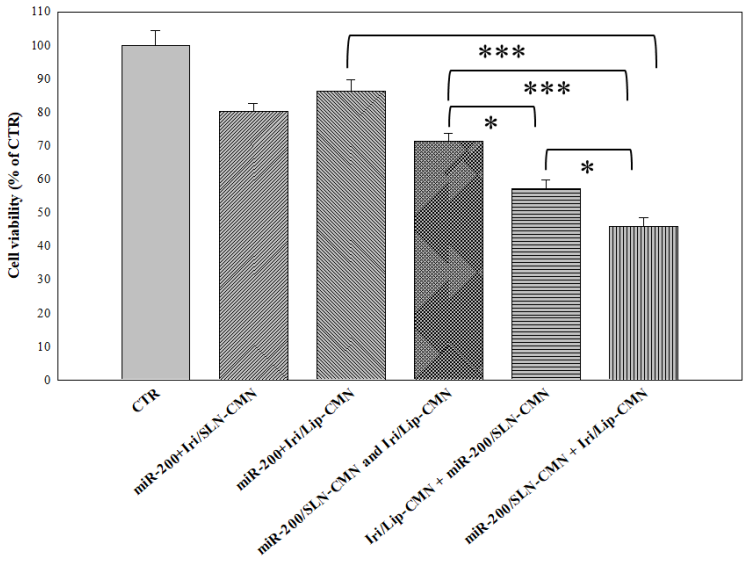

Figure S5. Evaluation of treatment order on cytotoxicity of different formulations in SAS cells for 48h by sulforhodamine B assay. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

miR-200 + Iri/SLN-CMN: miR-200+ Iri together in SLN-CMN for 48 h;

miR-200 + Iri/Lip-CMN: miR-200+ Iri together in Lip-CMN for 48 h;

miR-200/SLN-CMN and Iri/Lip-CMN: miR-200/SLN-CMN and Iri/Lip-CMN together for 48 h;

Iri/Lip-CMN + miR-200/SLN-CMN: Iri/Lip-CMN for 24 h and followed by miR-200/SLN-CMN for 24 h;

miR-200/SLN-CMN + Iri/Lip-CMN: miR-200/SLN-CMN for 24 h and followed by Iri/Lip-CMN for 24 h.

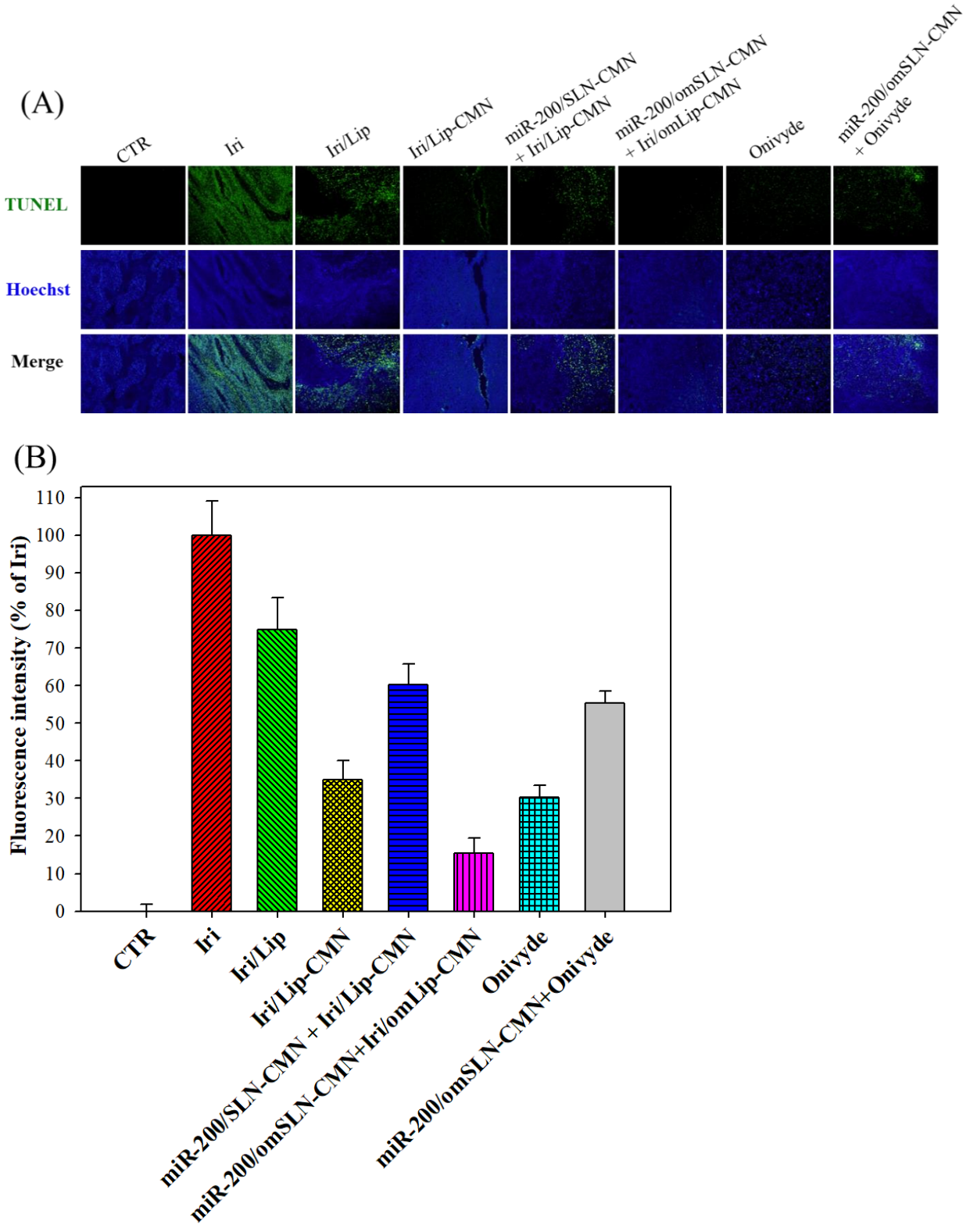

Figure S6. In vivo apoptosis evaluation in vessel cells of SAS/luc-bearing mice. (A) TUNEL analyses of *in vivo* apoptosis evaluation by different formulations on vessel cells of SAS/luc-bearing mice on the day after the last administration. Nuclei were stained with Hoechst (blue). Scale bar, 100 μm. (B) Relative apoptosis % of vessels cells was measured by the relative green fluorescence intensity of TUNEL assay.

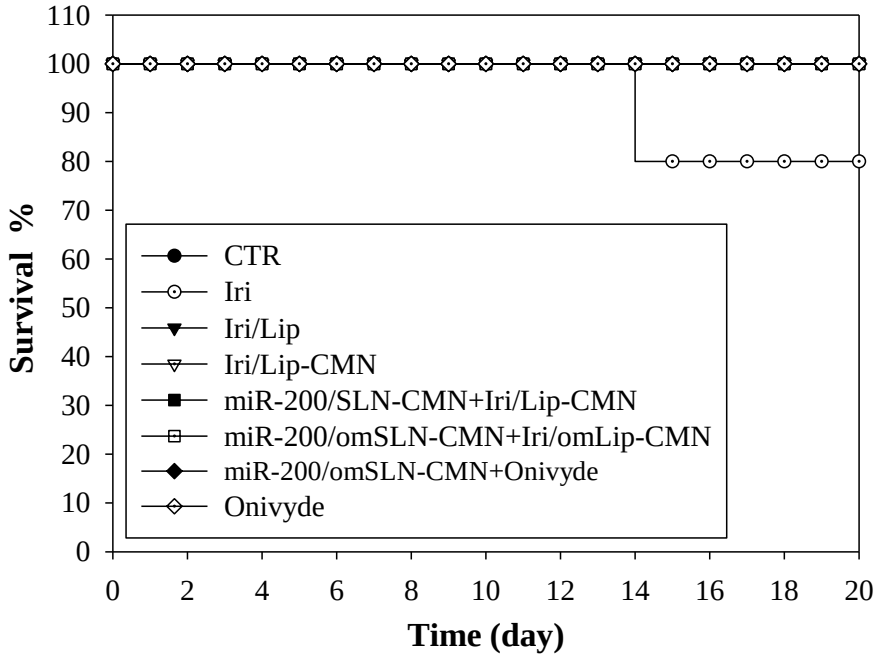

Figure S7. Survival % of SAS -bearing mice treated with different formulations at the final endpoint of the 20th day.