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Supplemental Information

Combination Cancer Therapy Using Chimeric Antigen Receptor-Engineered Natural Killer Cells as Drug Carriers

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SUPPLEMENTAL MATERIAL

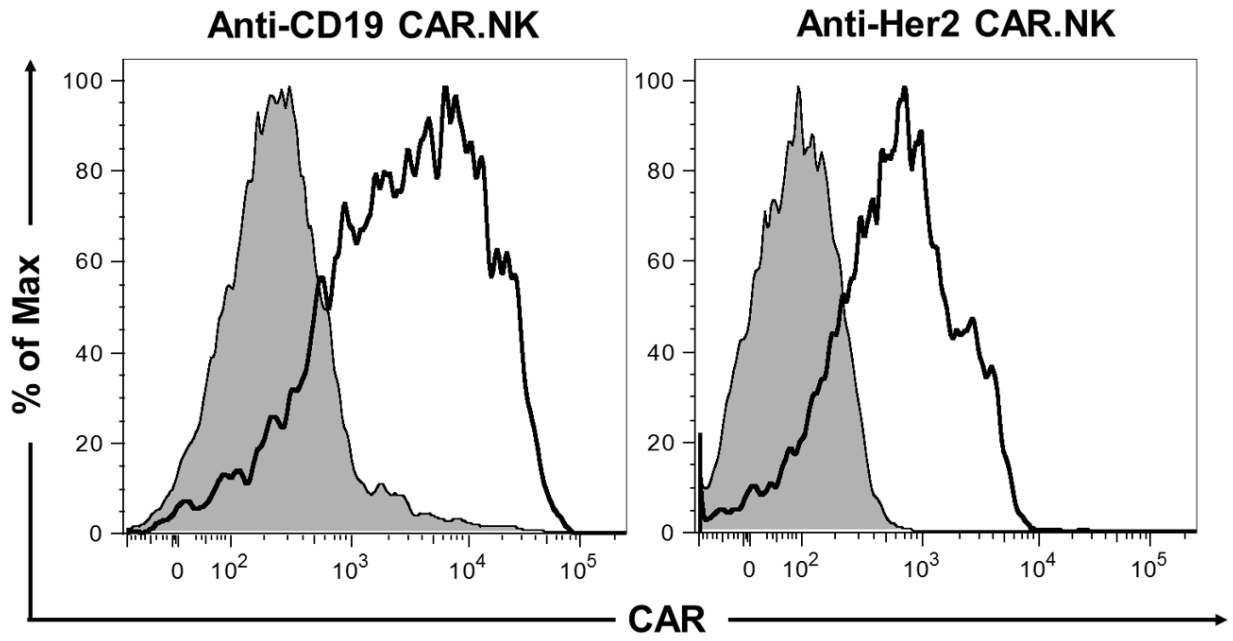

Figure S1. CAR expression in transduced NK cells. Nontransduced NK cells were used as a negative control (gray shaded peaks). Anti-CD19 CARs were detected using flow cytometry after being labeled with biotinylated Protein L and streptavidin conjugated to FITC. Anti-Her2 CAR.NK cells were detected with flow cytometry after being labeled with rhHer2-Fc chimera and PE-labeled goat anti-human Fc.

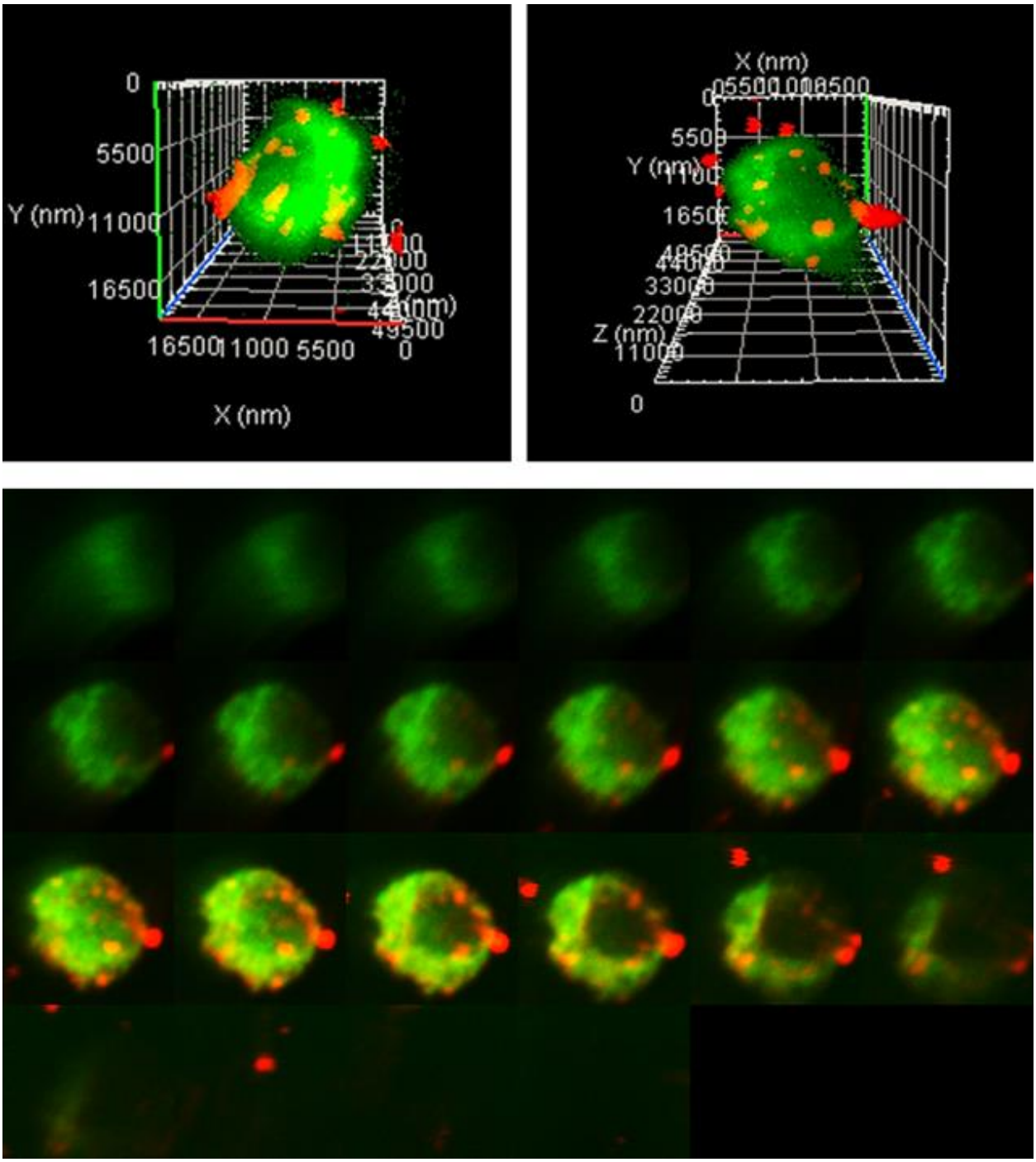

Figure S2. Confocal microscopy of CAR.NK cells conjugated to DiD-loaded cMLVs (cMLV(DiD))—3D and Z-stacked images. CAR.NK cells were labeled with 1 μM CFSE and washed with PBS prior to conjugation to cMLV(DiD).

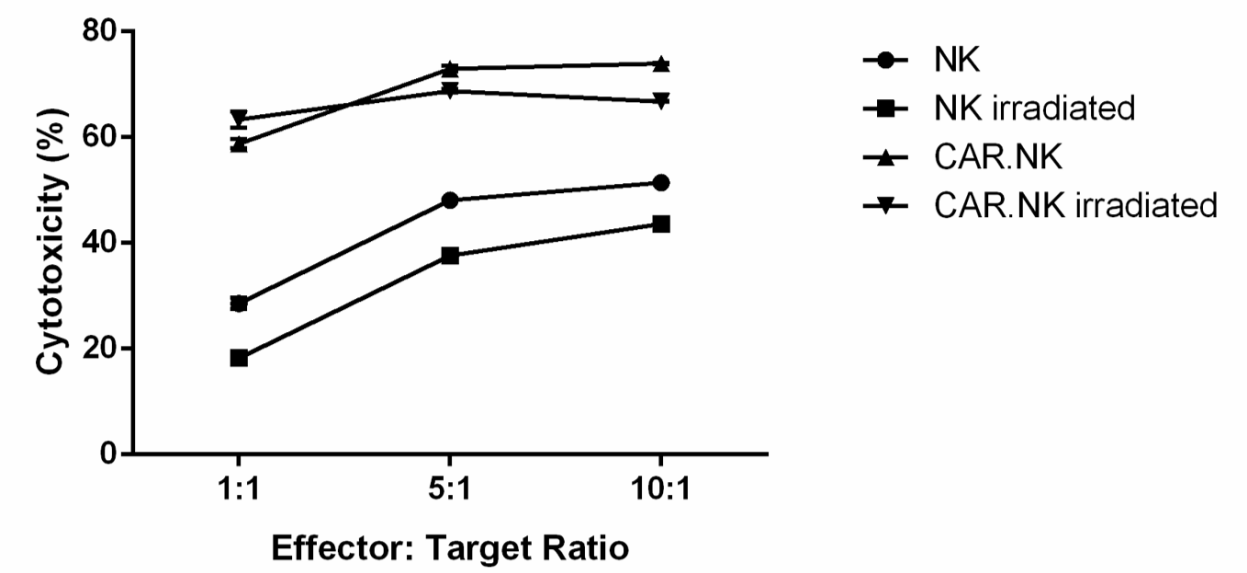

Figure S3. Cytotoxicity comparison between irradiated (5 Gy) and nonirradiated CAR.NK cells. NK or CAR.NK cells were cocultured with SKOV.CD19 cells for 24 hours at 1:1, 5:1, or 10:1 effector-to-target ratios and cytotoxicity was measured.

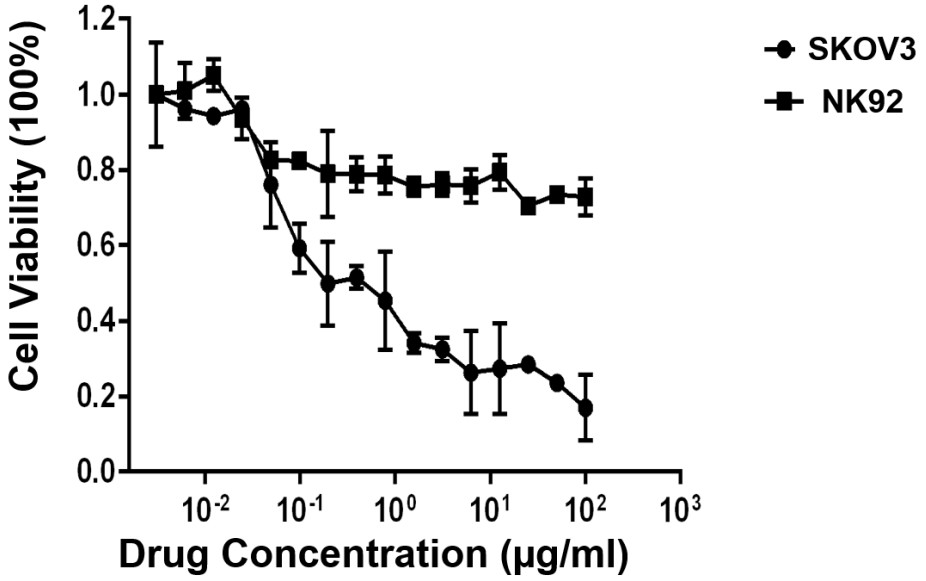

Figure S4. Cell viability assay with NK and SKOV3 cells exposed to PTX. Cells were incubated with various concentrations of cMLV(PTX). Cell viability percentage was determined by subtracting absorbance values obtained from media-only wells from the treated wells and then normalized by the control wells containing cells without drugs.

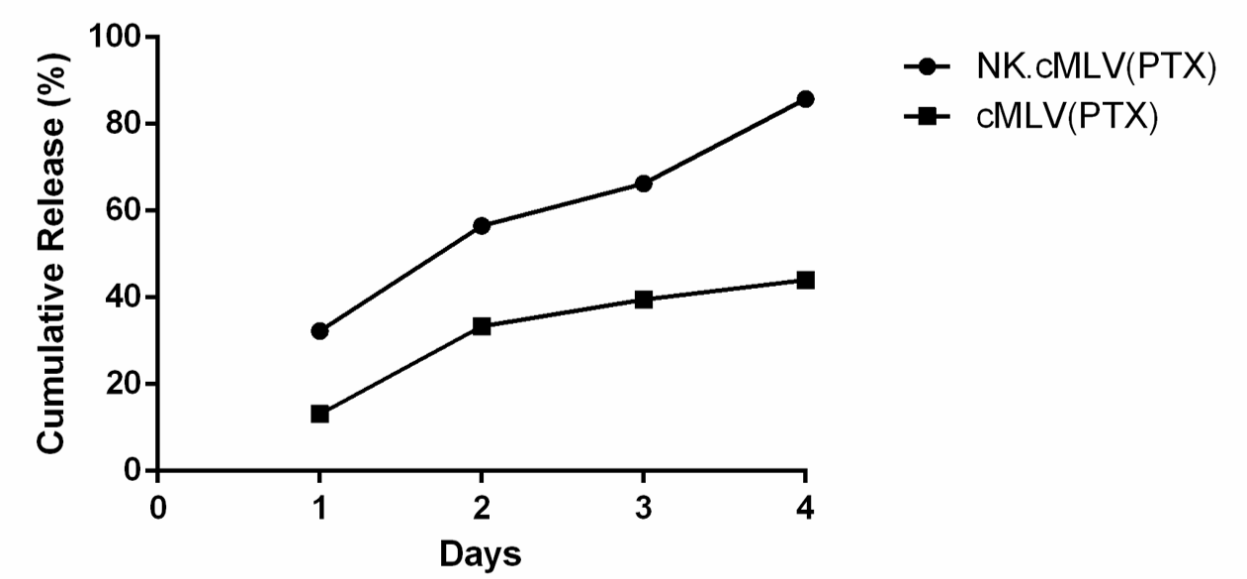

Figure S5. PTX release kinetics from free cMLVs and CAR.NK.cMLVs. To obtain the release kinetics of PTX from cMLVs before and after cell conjugation, cMLV(PTX) and CAR.NK.cMLV(PTX) were incubated in 10% FBS-containing media at 37 °C and were spun down and resuspended with fresh media daily. The PTX was quantified from the removed media by HPLC every day.