

Supplementary Materials

Liposomal Delivery of MIW815 (ADU-S100) for Potentiated STING Activation

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Table S1. Size distribution and zeta potential of liposomal ADU-S100 formulations with varying amounts of DOTAP and PEGylation. Results are presented as the mean \pm SD (n \geq 3).

Formulation	DOTAP: HSPC: Cholesterol: PEG ₂₀₀₀ -DSPE (mol%)	N/P ratio	Size (nm), PDI	Zeta potential (mV)
F1	45:0:50:5	20:1	102.62 \pm 3.87 (0.18 \pm 0.01)	13.73 \pm 1.19
F2	34:11:50:5	15:1	105.30 \pm 3.10 (0.17 \pm 0.02)	12.53 \pm 1.62
F3	22:23:50:5	10:1	100.70 \pm 6.77 (0.15 \pm 0.05)	12.00 \pm 2.56
F4	11:34:50:5	5:1	91.39 \pm 9.62 (0.16 \pm 0.02)	9.16 \pm 2.90
F5	6:39:50:5	2.5:1	87.44 \pm 7.72 (0.16 \pm 0.03)	5.24 \pm 3.11
F6	34:11:50:0	15:1	85.34 \pm 6.48 (0.25 \pm 0.03)	46.80 \pm 3.97
F7	34:11:50:10	15:1	92.43 \pm 6.43 (0.13 \pm 0.02)	9.07 \pm 3.00
F8 (MIX lipo)	34:11:50:5	15:1	98.30 \pm 3.70 (0.14 \pm 0.02)	7.73 \pm 1.82
F9 (blank lipo)	34:11:50:5	15:1	96.50 \pm 4.10 (0.15 \pm 0.02)	11.83 \pm 1.42

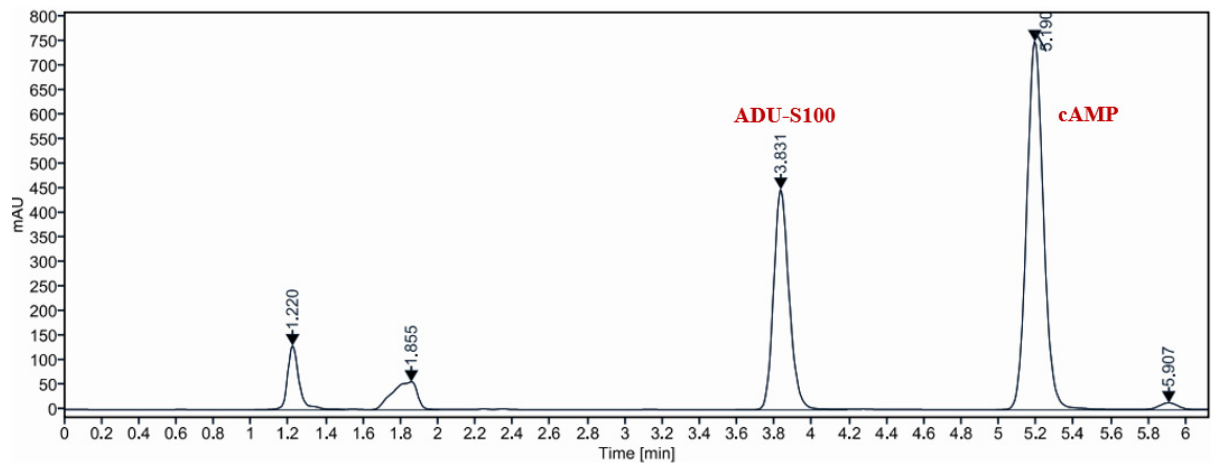


Figure S1. HPLC chromatogram of ADU-S100 and cAMP (internal standard).

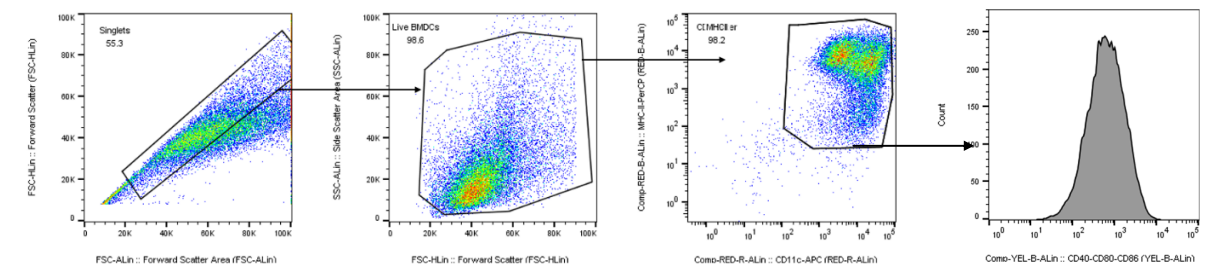


Figure S2. Flow cytometry gating strategy for analyzing BMDCs and the expression of co-stimulatory molecules.

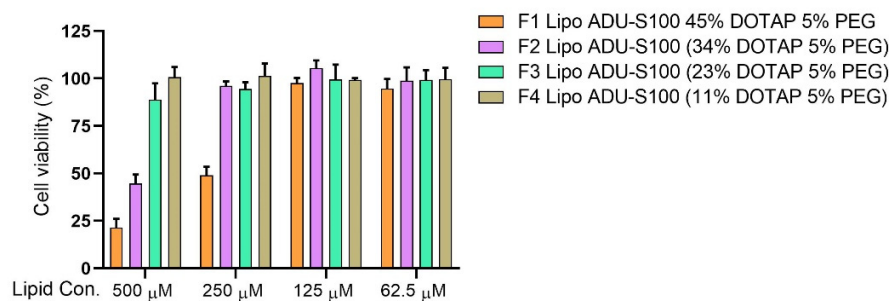


Figure S3. Cell viability assays of liposomal ADU-S100 with varying levels of DOTAP. Human lung carcinoma A549 cells were treated with liposomal ADU-S100 formulations at four lipids concentrations (500, 250, 125, 62.5 μ M), which corresponded to four ADU-S100 concentrations (4, 2, 1, 0.5 μ g/ml). After a 48-h incubation, the cell viability assay was measured by CyQUANT XTT cell viability kit (Invitrogen) according to the manufacturer's protocol.

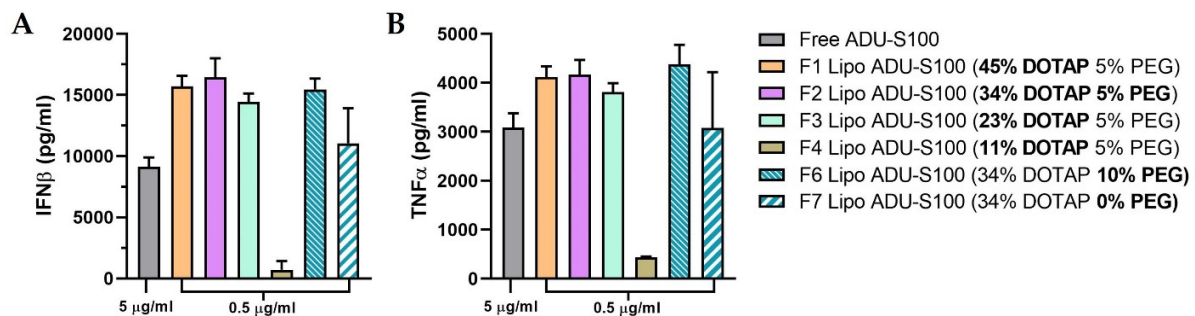


Figure S4. Production of TNF α (A) and IFN β (B) in BMDCs after stimulation with free ADU-S100 (5 μ g/ml) or liposomal ADU-S100 (0.5 μ g/ml) for 24 h. Data are shown as the mean \pm SD ($n \geq 3$) and are representative of three independent experiments.

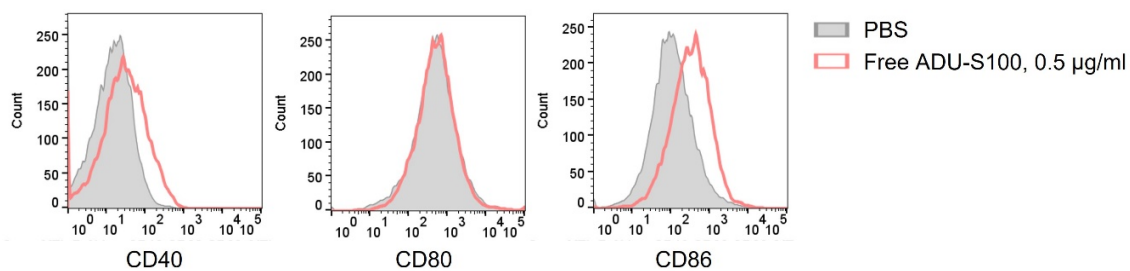


Figure S5. Representative flow plots of CD40, CD80, and CD86 expression after stimulation of BMDCs with PBS and free ADU-S100 (0.5 μ g/ml) for 6 h. Data are representative of three independent experiments.