

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

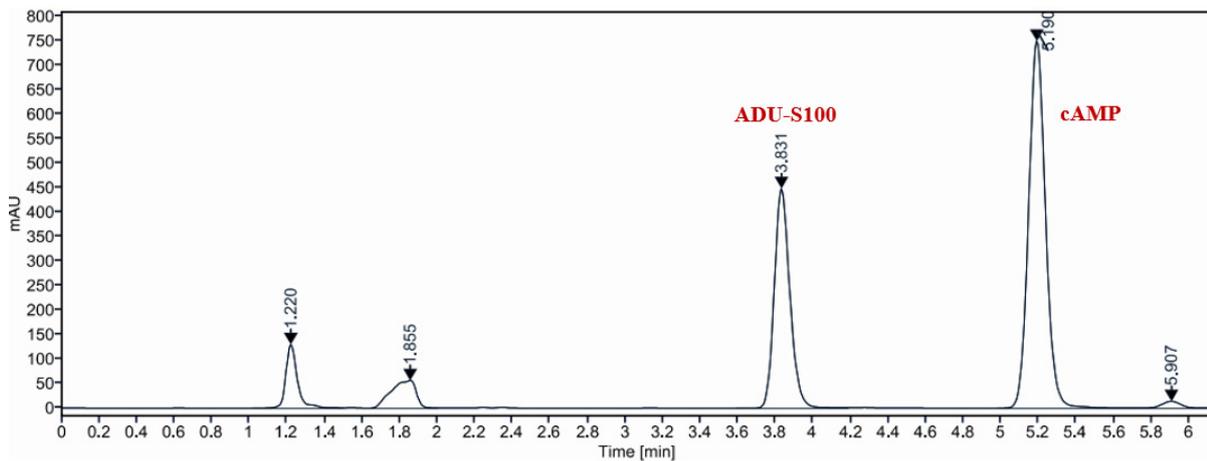


Figure S1. HPLC chromatogram of ADU-S100 and cAMP (internal standard).

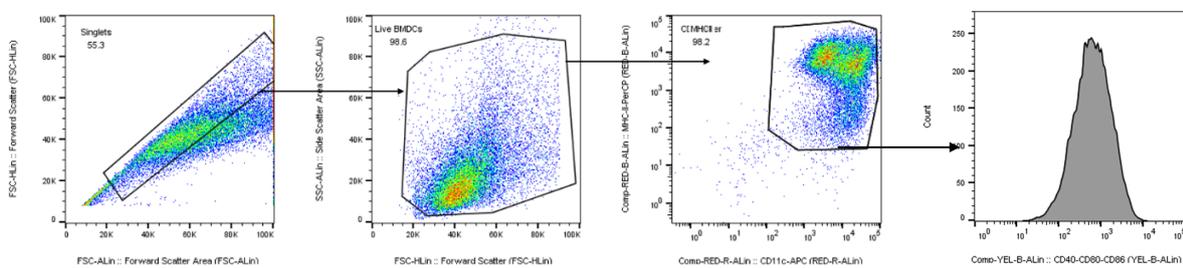


Figure S2. Flow cytometry gating strategy for analyzing BMDCs and the expression of co-stimulatory molecules.

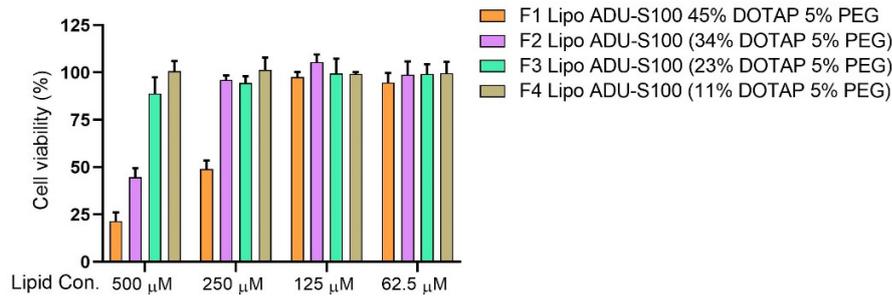


Figure S3. Cell viability assays of liposomal ADU-S100 with varying levels of DOTAP. Human lung carcinoma A549 cells were treated with liposomal ADU-S100 formulations at four lipid concentrations (500, 250, 125, 62.5 μM), which corresponded to four ADU-S100 concentrations (4, 2, 1, 0.5 μg/ml). After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

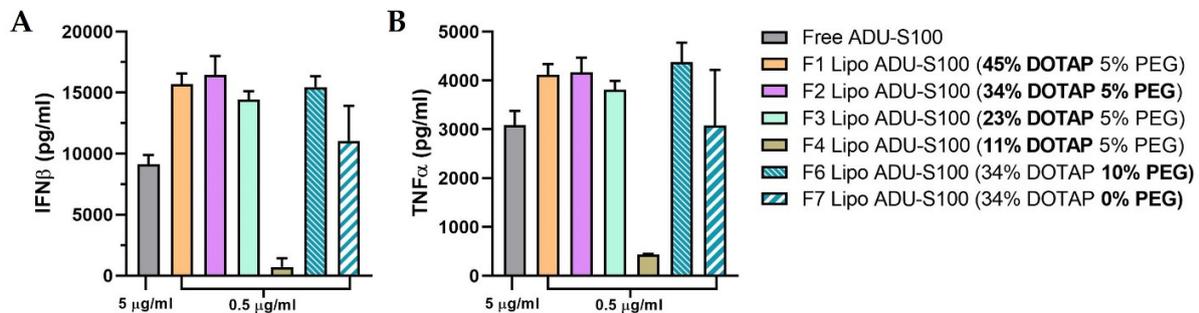


Figure S4. Production of TNFα (A) and IFNβ (B) in BMDCs after stimulation with free ADU-S100 (5 μg/ml) or liposomal ADU-S100 (0.5 μg/ml) for 24 h. Data are shown as the mean ± SD (n ≥ 3) and are representative of three independent experiments.

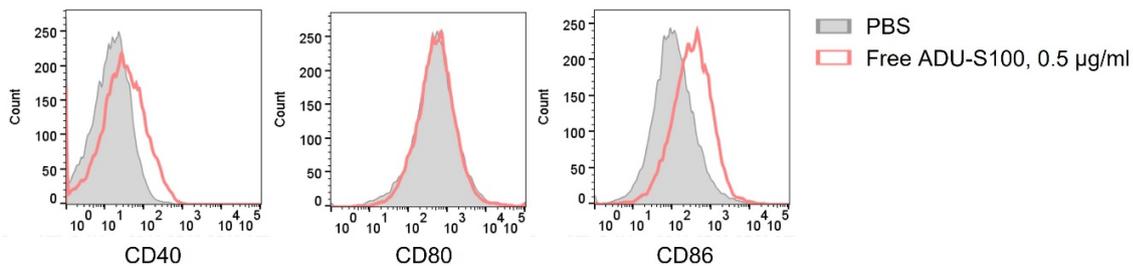


Figure S5. Representative flow plots of CD40, CD80, and CD86 expression after stimulation of BMDCs with PBS and free ADU-S100 (0.5 μg/ml) for 6 h. Data are representative of three independent experiments.