Supporting Information for

Unnatural Lipids for Simultaneous mRNA Delivery and Metabolic Cell Labeling

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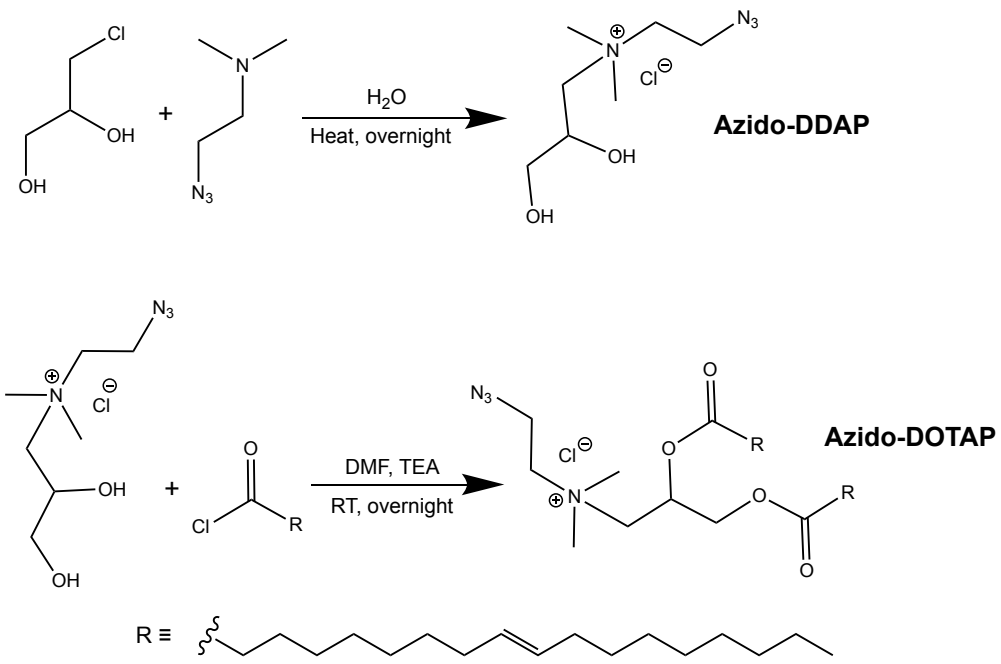

Figure S1. Synthetic route of azido-DDAP and azido-DOTAP.

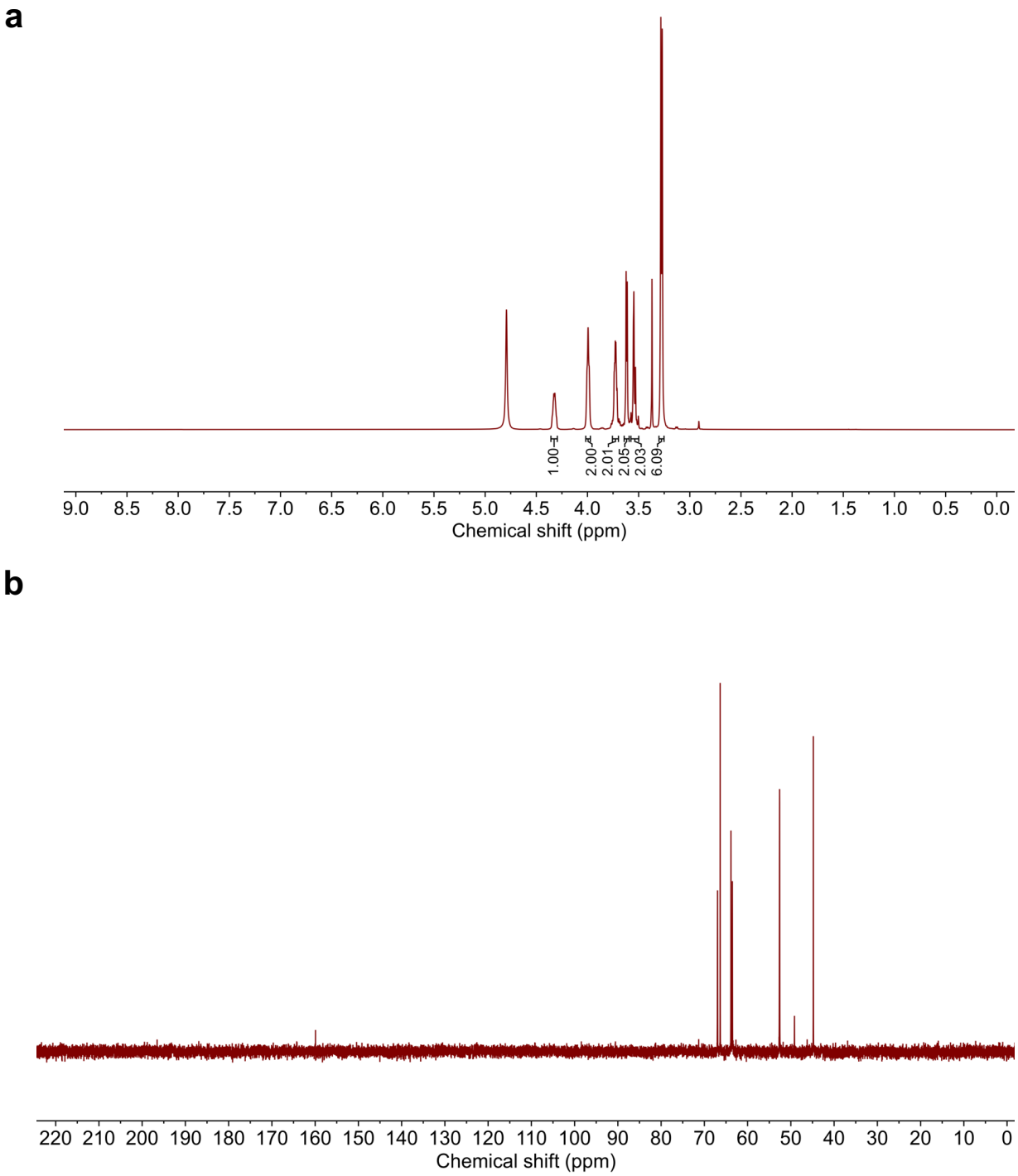

Figure S2. (a) ^{1}H NMR spectrum and (b) ^{13}C NMR spectrum of azido-DDAP in D_2O.

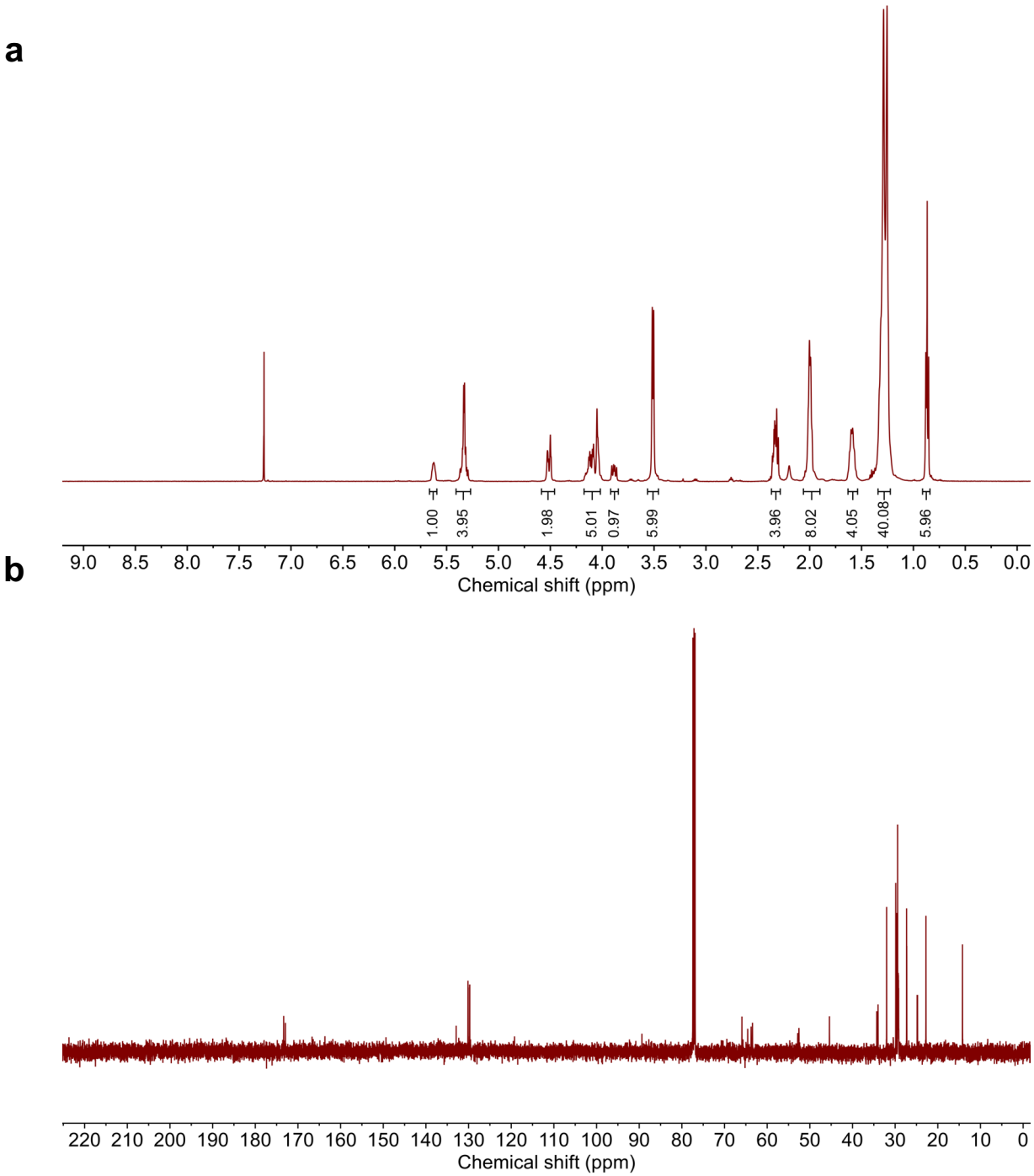

Figure S3. (a) ^{1}H NMR spectrum and (b) ^{13}C NMR spectrum of azido-DOTAP in $CDCl_3$.

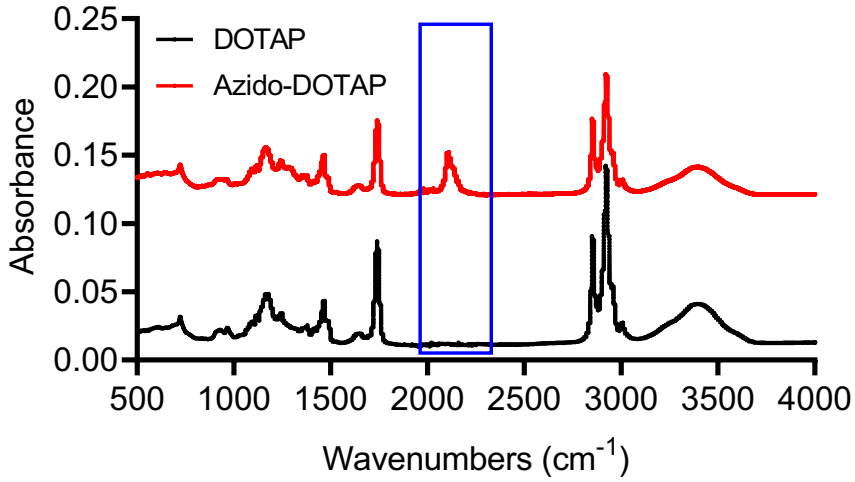

Figure S4. FTIR spectra of DOTAP and azido-DOTAP. The peak at 2106 cm^{-1} indicates the presence of azido groups.

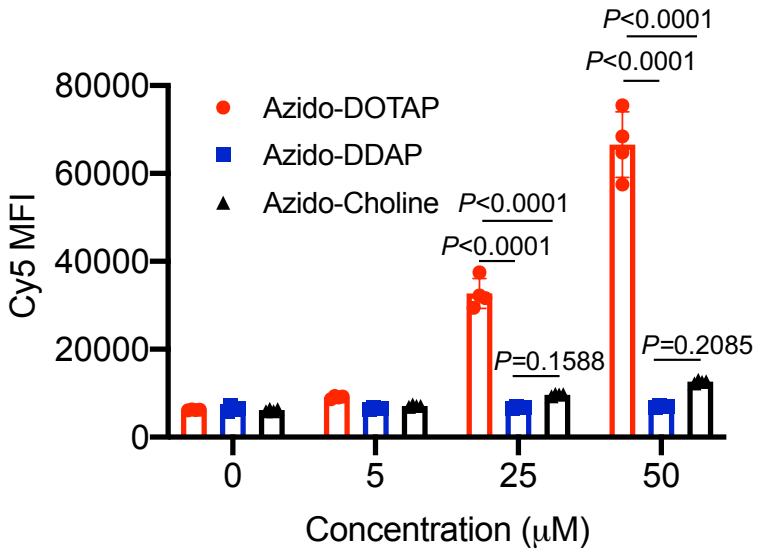

Figure S5. Cy5 fluorescence intensity of 4T1 cells after 24-h treatment with azido-DOTAP, azido-DDAP, or azido-choline (0, 25, and 50 μM, respectively) and 20-min staining with DBCO-Cy5. All the numerical data are presented as mean ± SD ($0.01 < *P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$).

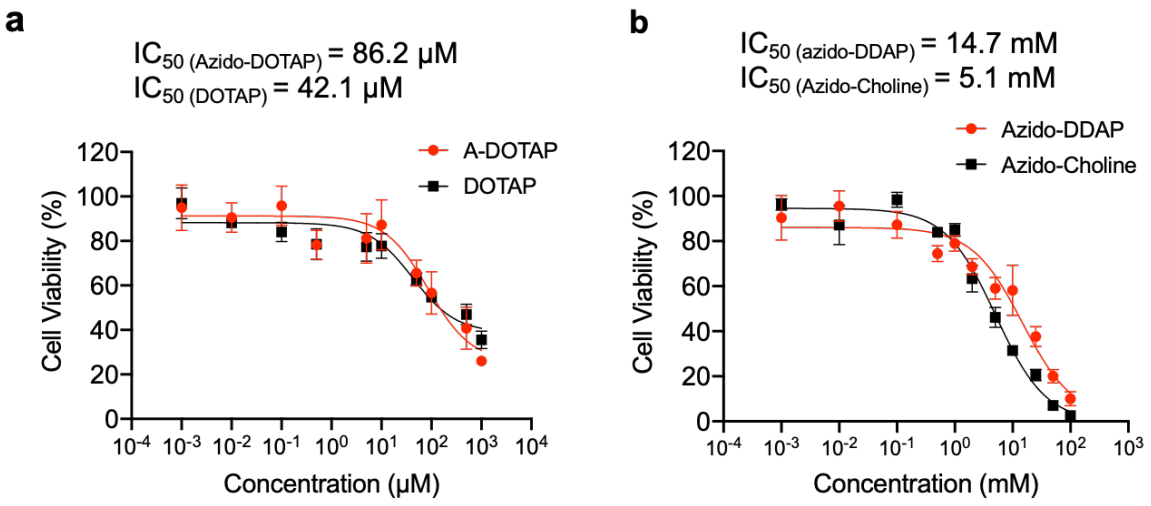

Figure S6. Viability of 4T1 cells after treatment with different concentrations of (a) azido-DOTAP, DOTAP, (b) azido-DDAP, or azido-Choline for 72 h, as measured via an MTT assay.

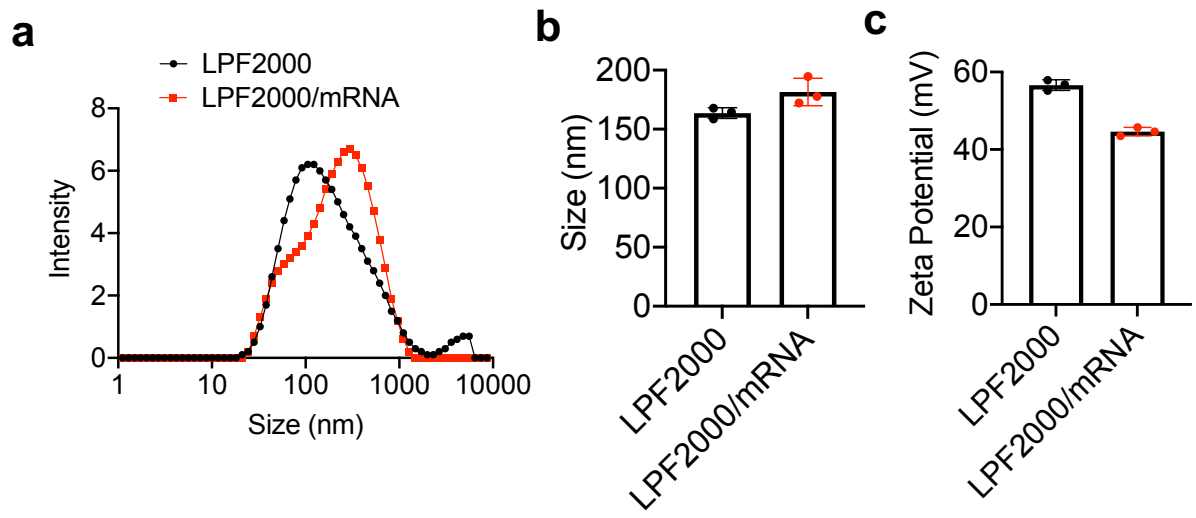

Figure S7. (a) Representative dynamic light scattering (DLS) profile of LPF2000 nanoparticles and LPF2000/mRNA complexes. (b) Average size of LPF2000 nanoparticles and LPF2000/mRNA complexes. (c) Zeta potential of LPF2000 nanoparticles and LPF2000/mRNA complexes. The mass ratio of LPF2000 and mRNA was fixed at 5:1.

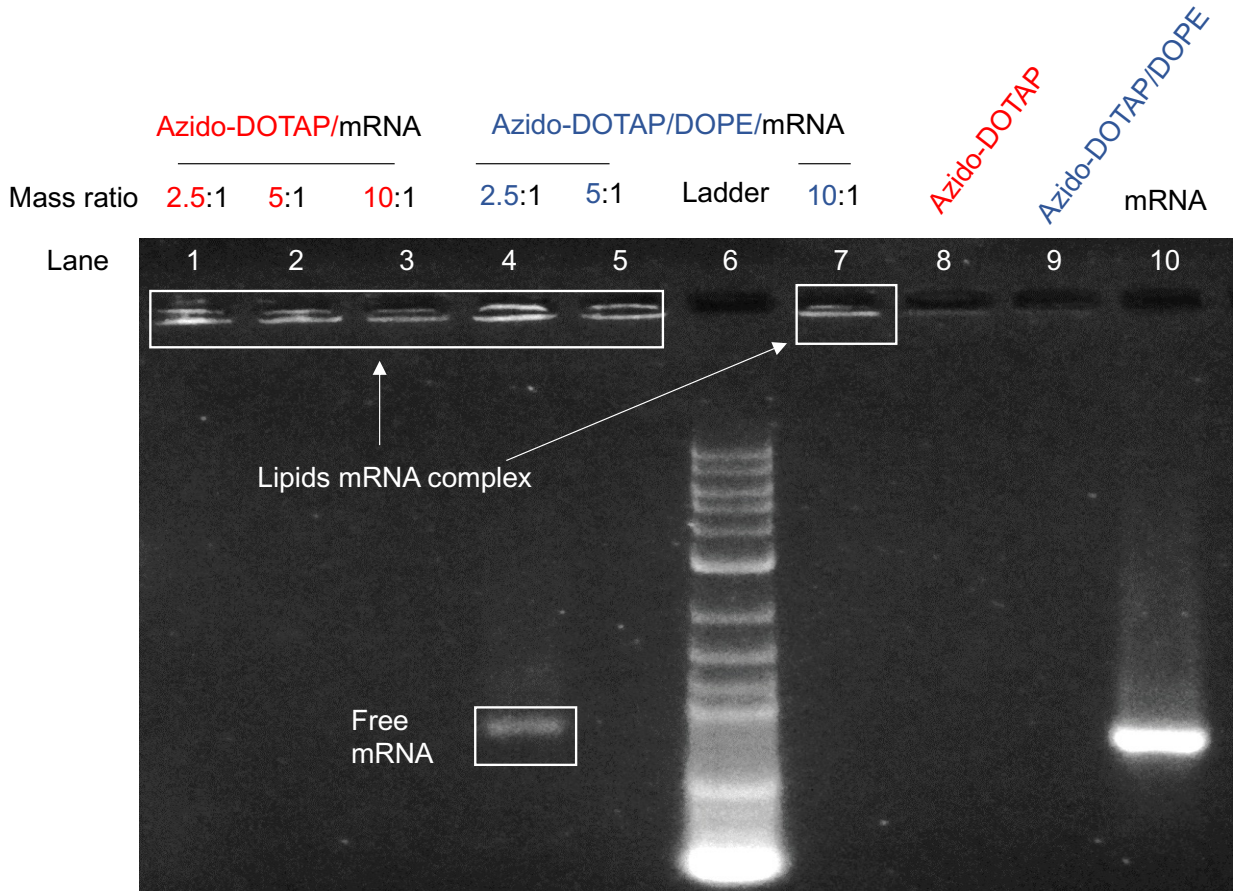

Figure S8. Agarose gel electrophoresis of azido-DOTAP/mRNA and azido-DOTAP/DOPE/mRNA complexes. Different mass ratios of lipids and eGFP mRNA were mixed in the Opti-MEM medium and incubated for 30 minutes prior to gel running. For azido-DOTAP/DOPE/mRNA complexes, the molar ratio of azido-DOTAP and DOPE was set at 6:4. Free mRNA, azido-DOTAP, and azido-DOTAP/DOPE mixture were used as controls. The amount of mRNA was fixed at 100 ng per lane. Note that 10:1 mass ratio equals to 4.5 N/P ratio.

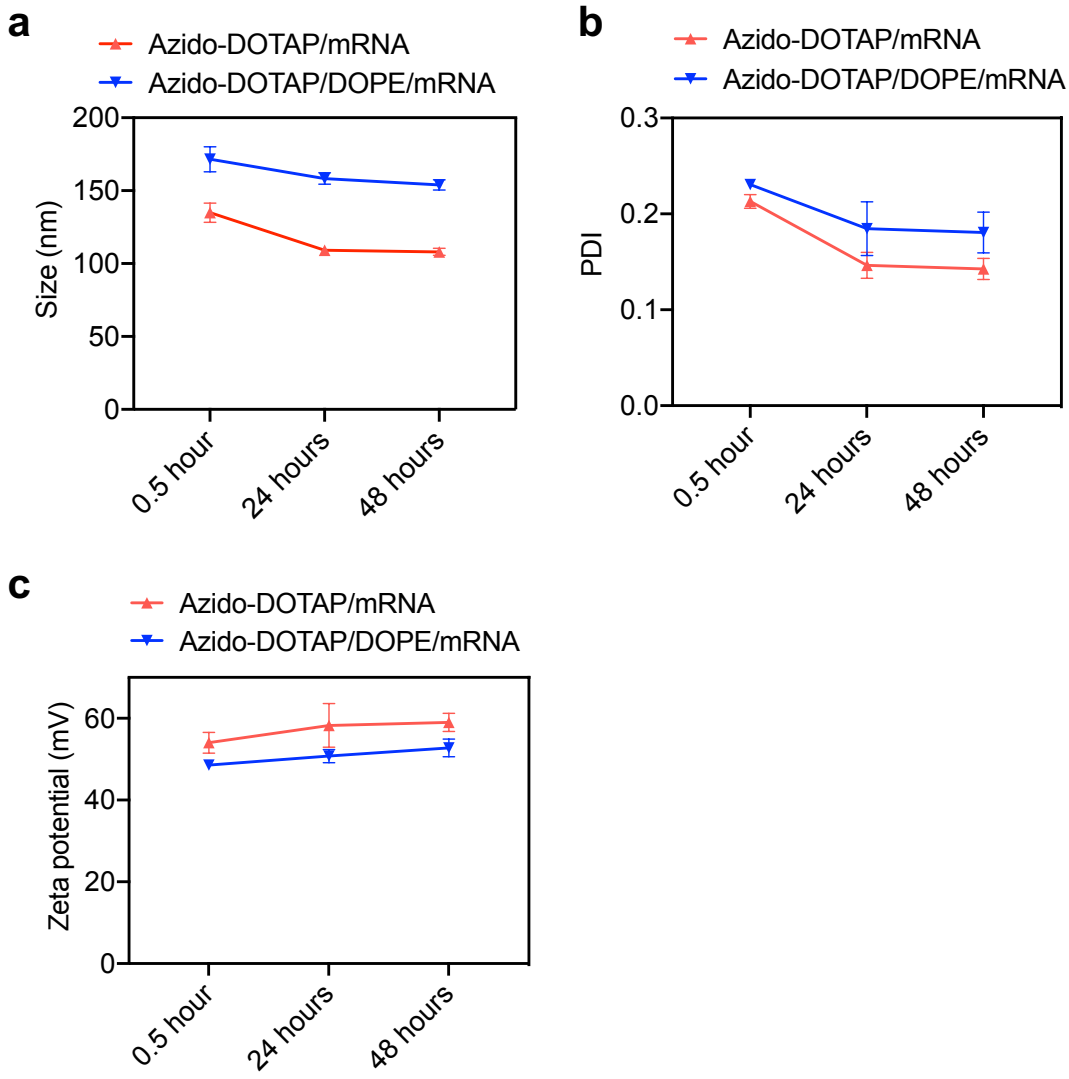

Figure S9. (a) Average size, (b) PDI, and (c) zeta potential of azido-DOTAP/mRNA and azido-DOTAP/DOPE/mRNA complexes after incubation at 37℃ for 0.5, 24, and 48 hours, respectively. The mass ratio of LPF2000 and mRNA was fixed at 5:1. For azido-DOTAP/DOPE/mRNA complexes, the molar ratio of azido-DOTAP and DOPE was set at 6:4.

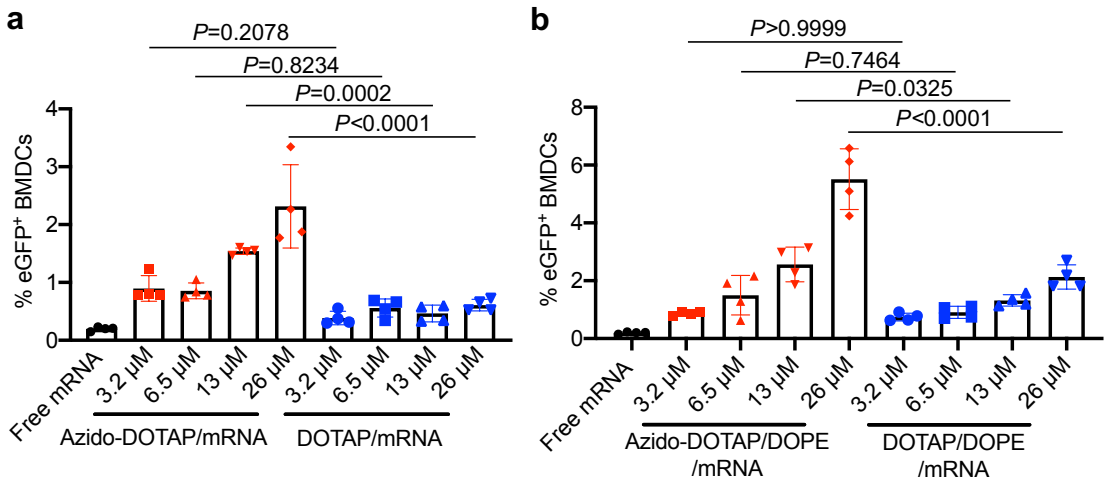

Figure S10. (a) % eGFP$^+$ BMDCs after 48-h treatment with free mRNA, azido-DOTAP/mRNA, or DOTAP/mRNA. (b) % eGFP$^+$ BMDCs after 48-h treatment with free mRNA, azido-DOTAP/DOPE/mRNA, or DOTAP/DOPE/mRNA. The concentration of mRNA was fixed at 1 μg/mL, while the concentration of azido-DOTAP or DOTAP was 3.2, 6.5, 13, and 26 μM, respectively. The molar ratio of azido-DOTAP (or DOTAP) to DOPE was 9:1. All the numerical data are presented as mean ± SD ($0.01 < *P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$).

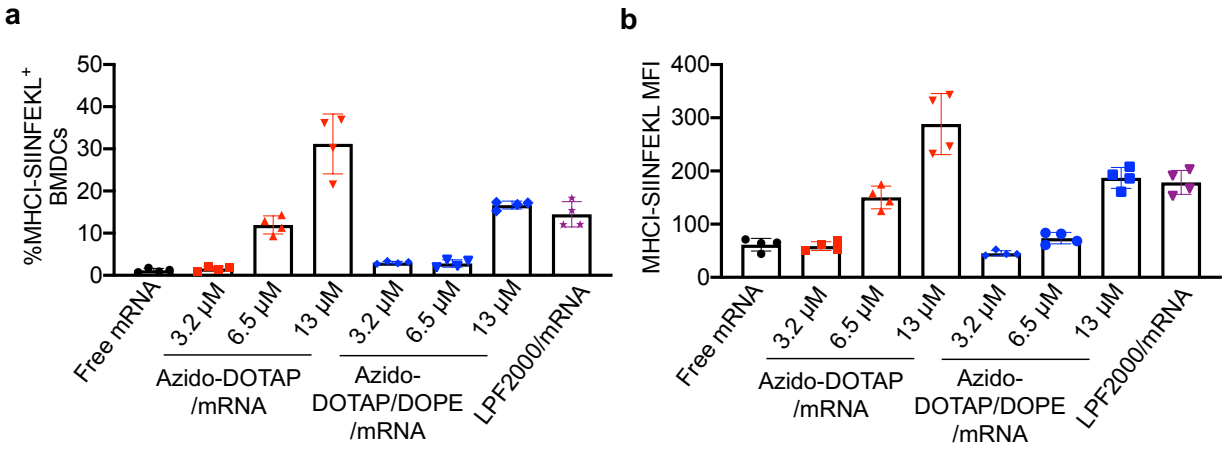

Figure S11. (a) Percentages of MHCI-SIINFEKL$^+$ BMDCs and (b) mean fluorescence intensity of BMDCs after incubating BMDCs with free mRNA, azido-DOTAP/mRNA, azido-DOTAP/DOPE/mRNA, or LPF2000/mRNA for 24 h. Cells were stained with APC-conjugated anti-MHCI-SIINFEKL, prior to flow cytometry analysis. All the numerical data are presented as mean ± SD ($0.01 < *P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$).

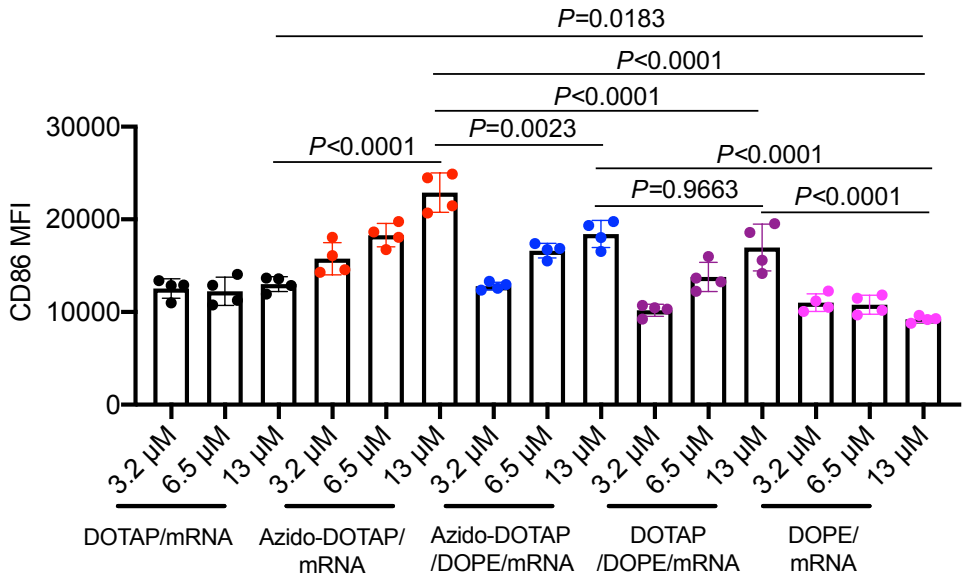

Figure S12. Mean CD86 fluorescence intensity of BMDCs after 48-h treatment with different groups and staining with PE/Cy7-conjugated anti-CD86. The concentration of SIINFEKL mRNA was fixed at 1 μg/mL, while the concentration of azido-DOTAP or DOTAP was 3.2, 6.5, 13, and 26 μM, respectively. The molar ratio of azido-DOTAP (or DOTAP) to DOPE was 9:1. All the numerical data are presented as mean ± SD ($0.01 < *P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$).