

Design of lipid nanoparticles with potent reactive oxygen species scavenging capacity for efficient mRNA delivery

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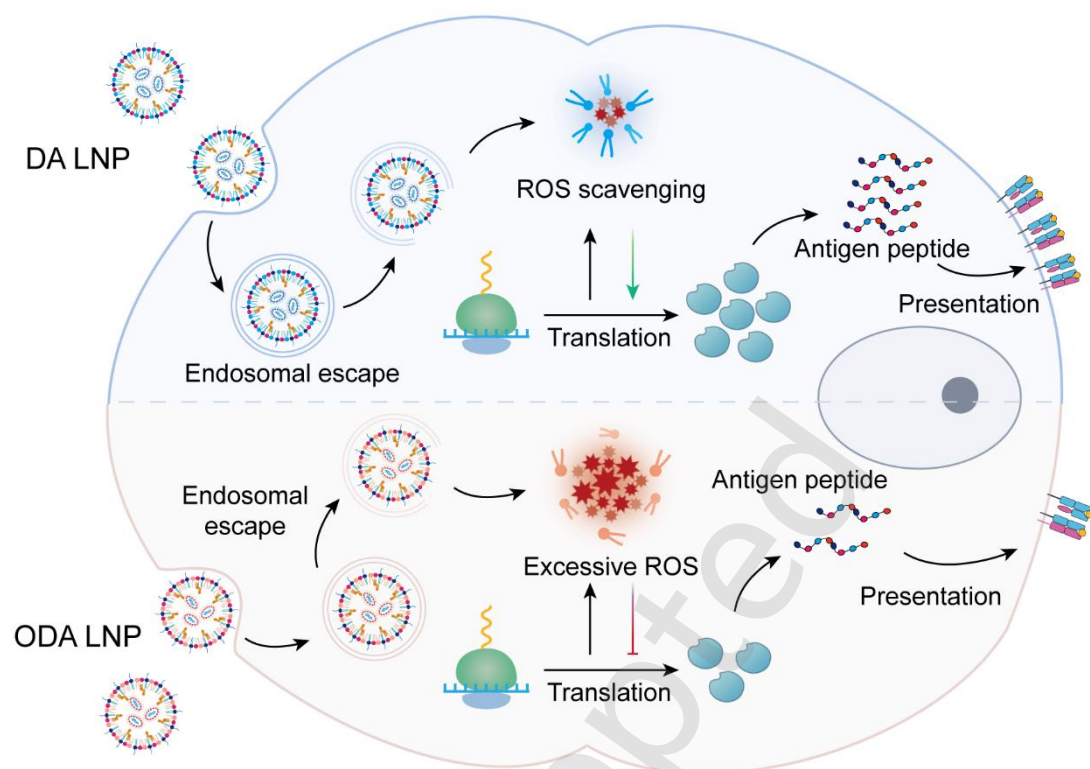
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A dopamine-derived antioxidant lipid nanoparticle (DA LNP) scavenges intracellular ROS, relieving oxidative stress and thereby improving mRNA-derived protein expression and antitumor immune protection in a prophylactic melanoma model.

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ABSTRACT: Lipid nanoparticles (LNPs) have become a clinically established platform for mRNA vaccine delivery, demonstrating significant potential in both infectious disease and cancer immunotherapy. However, excessive intracellular reactive oxygen species (ROS) can impair protein synthesis and compromise vaccine efficacy. To address this limitation, we developed an ionizable lipid incorporating a dopamine-derived moiety (DA lipid) for the formulation of ROS-scavenging LNPs. Comparative studies with oxidized control LNPs (ODA LNPs) revealed that DA LNPs exhibit superior intrinsic antioxidant activity, effectively mitigating intracellular ROS accumulation while supporting higher mRNA-derived protein expression. DA LNPs mediated transfection levels comparable to those achieved with ALC-0315 LNPs (ALC LNPs). In addition, DA LNPs induced stronger antigen-specific immune responses and more potent tumor suppression in a prophylactic mouse melanoma model compared to ODA LNPs. These results support the importance of redox modulation in optimizing mRNA vaccine performance and provide a rational design strategy for advanced nucleic acid delivery systems.

KEYWORDS: lipid nanoparticles, reactive oxygen species, mRNA delivery, dopamine

1 Introduction

mRNA-based vaccines have emerged as a versatile and powerful platform for disease prevention and treatment, most notably exemplified by their rapid development and deployment during the COVID-19 pandemic[1-6]. In contrast to conventional vaccine technologies, mRNA vaccines offer numerous advantages including rapid design cycles, scalable manufacturing processes, and the ability to encode complex antigenic proteins[7-10]. The clinical translation of mRNA vaccines has been largely enabled by critical advances in lipid nanoparticle (LNP) delivery systems[2, 11-15], which serve to protect mRNA from degradation, facilitate cellular uptake, and promote efficient endosomal escape for successful translation[16-20].

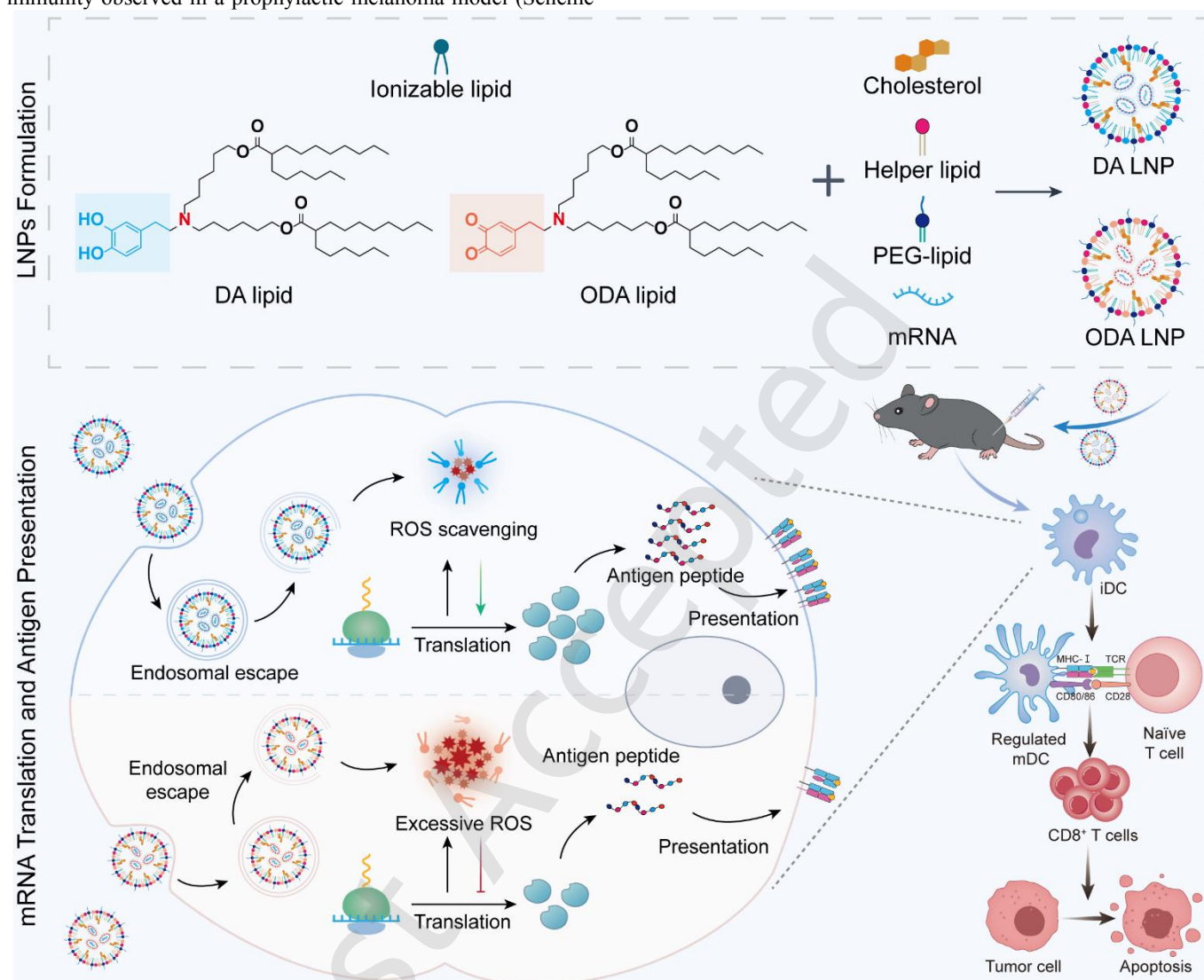
A significant yet underexplored challenge in mRNA vaccine technology involves reactive oxygen species (ROS) accumulation associated with cellular stress responses during translation, where excessive ROS accumulation induces oxidative stress and cellular damage, activating hypoxia-response pathways that suppress protein synthesis, ultimately diminishing vaccine immunogenicity[21-28]. Oxidative stress can inhibit translation initiation and impair translational fidelity, while NAC has been reported to restore translational fidelity in an oxidative-stress-sensitive cellular

model[29, 30]. Previous approaches to mitigate ROS-related effects have primarily involved the incorporation of exogenous antioxidants or the design of polymeric vectors with intrinsic radical-scavenging motifs[27, 31, 32]. However, the development of ionizable lipids that effectively combine ROS scavenging capability with efficient mRNA delivery remains substantially limited. Recently, trisulfide-derived ROS-scavenging LNPs were used to deliver IL-4 mRNA for diabetic wound healing[33]. Antioxidant and redox-adaptive ionizable lipids have been shown to preserve mRNA integrity, support mRNA-derived protein expression, and enhance mRNA vaccine potency[34, 35]. Here, a catechol antioxidant motif was integrated directly into an ionizable lipid to examine how redox regulation affects mRNA-derived protein expression and antitumor vaccination.

Dopamine is an endogenous catecholamine neurotransmitter. Its catechol moiety confers potent antioxidant activity, while the primary amine group remains chemically accessible and reactive. This dual-functional molecular structure makes dopamine a promising scaffold for the rational design of ionizable lipid[36-41]. We report herein the rational synthesis of a novel dopamine-derived ionizable lipid (DA lipid) and its oxidized analogue (ODA lipid) for LNP formulation. The resulting DA LNPs maintain potent antioxidant capacity while exhibiting efficient mRNA delivery performance.

In this study, we systematically demonstrate that DA LNPs combine intrinsic ROS-scavenging activity with efficient mRNA delivery, resulting in enhanced mRNA-derived protein expression and antigen presentation both *in vitro* and *in vivo*. These effects may contribute to the improved antitumor immunity observed in a prophylactic melanoma model (Scheme

1). This work presents an innovative lipid design strategy that successfully integrates intrinsic antioxidant functionality with efficient nucleic acid delivery, and establishes a foundation for developing advanced delivery systems for nucleic acid-based therapeutics.



Scheme 1 Schematic illustration of dopamine-derived ionizable lipid nanoparticles (DA LNPs) and the oxidized counterparts (ODA LNPs) for mRNA delivery and immunotherapy.

2 Results and discussion

2.1 Synthesis and Physicochemical Characterization

The DA lipid was synthesized through conjugation of a dopamine-derived headgroup with a hydrophobic tail composed of 2-hexyldecanoic acid connected via a C6 alkyl spacer. Chemical oxidation of DA lipid yielded the ODA lipid, enabling direct comparison of the impact of catechol integrity on LNP performance. Structural validation was confirmed through comprehensive characterization using ^1H NMR and mass spectrometry (Figs. S1–S7 in the Electronic Supplementary Material (ESM)). UV-Vis and FT-IR analyses provided conclusive evidence of catechol to quinone conversion in ODA lipid, demonstrated by a characteristic redshift in absorption spectra and the emergence of distinctive carbonyl stretching vibrations (Figs. S8 and S9 in the ESM). LNPs were formulated using rapid mixing technology with fixed molar ratios of ionizable lipid, cholesterol, DSPC, and DMG-PEG (50: 38.5: 10:

1.5). Systematic characterization revealed that DA LNPs exhibited a smaller mean diameter compared to both ODA LNPs and ALC LNPs (Fig. S10 in the ESM), a finding corroborated by TEM imaging (Fig. S11 in the ESM). This size reduction may be attributed to modified electrostatic repulsion dynamics during nanoparticle self-assembly, consistent with the measured surface charge characteristics of DA LNPs (Fig. S12 in the ESM). Both DA and ODA LNPs exhibited apparent pK_a values within the optimal range of 6.0–7.0 (Fig. S13 in the ESM), indicating preserved endosomal disruption potential. Agarose gel electrophoresis showed retardation of mRNA migration after formulation with DA or ODA LNPs (Fig. S14 in the ESM).

2.2 ROS Scavenging Capability and Transfection *in vitro*

The radical scavenging capacity of the synthesized lipids was quantitatively evaluated using established ABTS and pyrogallol autoxidation assays. DA lipid demonstrated concentration-dependent antioxidant activity superior to ODA lipid (Figs. 1(a) and 1(b)), and effectively neutralized DPPH

radicals in supplemental assays (Fig. S15 in the ESM). To investigate whether LNPs maintained this functionality in biological environments, we employed the DCFH-DA fluorescent probe for intracellular ROS detection. Confocal microscopy and flow cytometry results revealed substantially lower ROS levels in cells treated with DA LNPs compared to those treated with ODA LNPs or LPS stimulation (Figs. 1(c) and 1(d)).

To determine whether the lower ROS signal in DA-LNP-treated cells merely reflected reduced cellular activity or lower reporter-protein expression, we compared luciferase and GFP expression among the different formulations. Quantitative analysis demonstrated that DA LNPs mediated higher luciferase activity in DC2.4 cells compared to ODA LNPs and showed a similar level to ALC LNPs (Fig. 1(e)). A similar trend was observed for GFP expression (Fig. 1(f)).

Because oxidative stress can suppress protein synthesis[25, 26]^[25, 26], we examined whether reducing ROS independently of DA lipid chemistry affected mRNA-derived protein expression. Representative DCFH-DA fluorescence images showed lower ROS-associated fluorescence following NAC pretreatment, while NAC pretreatment increased luciferase expression from both ALC-0315 and SM102 based mRNA-LNPs, whereas GFP-positive cell percentages and mean fluorescence intensity changed only modestly (Figs. S16 and S17 in the ESM). At 1 mM, NAC did not reduce the CCK-8 signal relative to the 0 mM control, indicating that the NAC concentration used in the transfection experiments did not measurably decrease cell viability (Fig. S18 in the ESM). These results further support a relationship between lower intracellular ROS and increased mRNA-derived protein expression.

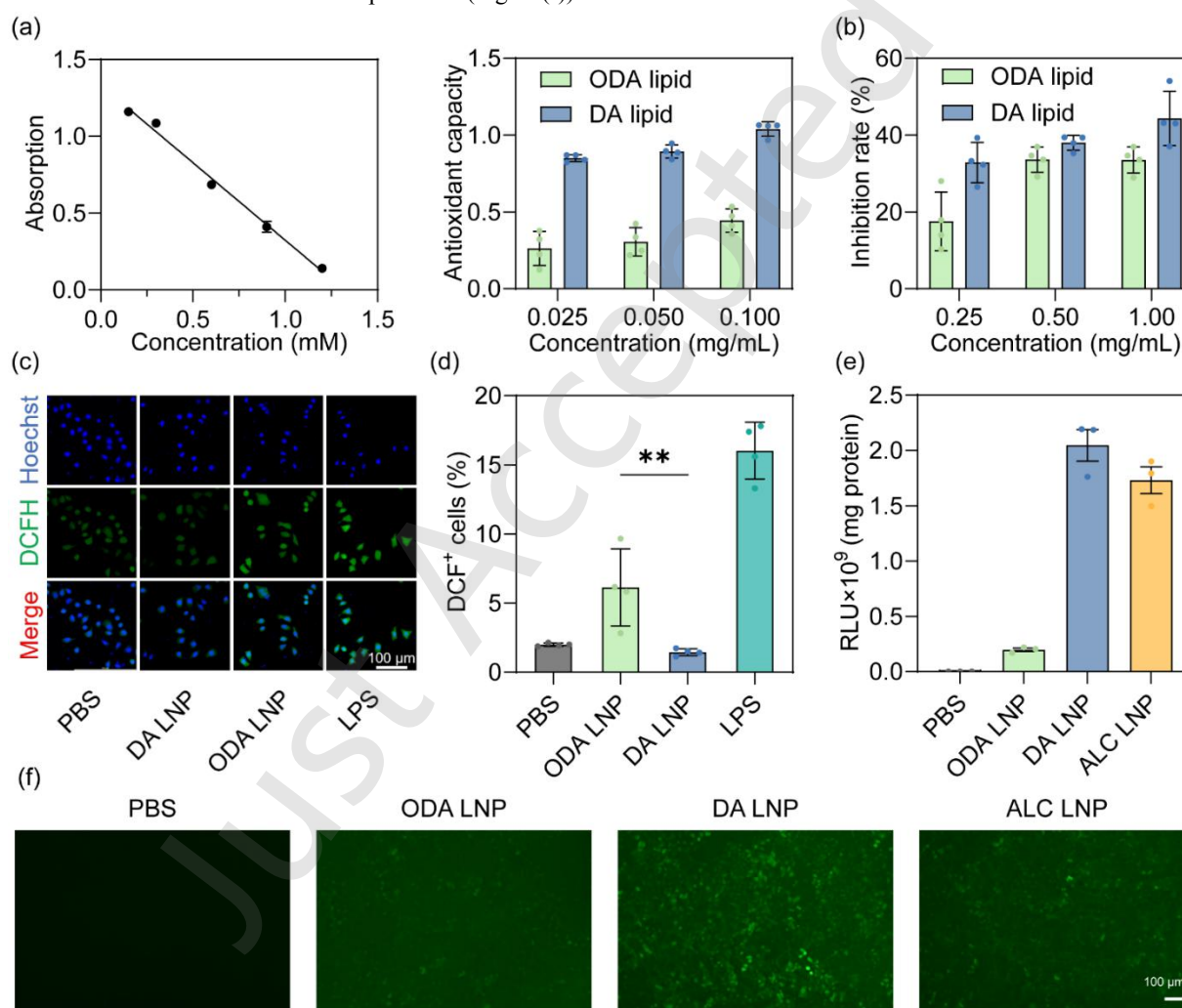


Figure 1 ROS-scavenging capacity of DA lipid and DA LNPs and transfection *in vitro*. (a) ABTS radical scavenging by DA and ODA lipid at various concentrations; Standard curve of the kit-provided antioxidant standard (left) and relative antioxidant capacities of ODA and DA lipid normalized to the standard (right; standard = 1, $n = 4$), DA lipid showed higher scavenging activity in a dose-dependent manner. (b) Superoxide scavenging assessed by pyrogallol autoxidation assay; DA lipid consistently reduced $\cdot\text{O}_2^-$ levels more effectively than ODA lipid ($n = 4$). (c) CLSM images of intracellular ROS levels in DC2.4 cells after various treatments for 6 h, measured using DCFH-DA as a ROS probe; DA LNPs reduced ROS compared with ODA LNPs. (d) Statistical FCM results of DCF⁺ DC2.4 cells after various treatments for 6 h ($n = 4$). (e) Luciferase expression *in vitro* following mRNA delivery by LNPs; DA LNPs produced higher luminescence than ODA LNPs and comparable to ALC LNPs ($n = 3$). (f) Representative images of GFP expression in DC2.4 cells using different LNPs. Data are presented as mean $\pm$ SD. Statistical significance was carried out by one-way ANOVA (** $p < 0.01$).

2.3 ROS Scavenging Capability and Transfection *in vivo*

Extended evaluation of *in vivo* ROS scavenging capacity was conducted through intramuscular administration of LNPs in mice. Tissue analysis performed 12 hours post-injection demonstrated that DA LNPs-treated mice exhibited significantly

reduced DCF fluorescence across multiple tissues, with particularly pronounced effects observed in spleen and lymph node (Figs. 2(a)-2(d)). These consistent findings confirm the systemically enhanced antioxidant capacity of DA LNPs in biologically relevant environments.

In vivo expression profiling following intramuscular administration of luciferase mRNA-loaded LNPs yielded enhanced and prolonged bioluminescence signals at injection sites and in organs for DA LNPs compared to ODA LNPs (Figs. 2(e), 2(f) and Fig. S19 in the ESM). Notably, DA LNPs reached a level of transfection efficiency comparable to that of ALC

LNPs. Following intramuscular administration of OVA mRNA-loaded LNPs, DA LNPs produced a modest increase in the proportion of splenic CD80⁺ CD86⁺ CD11c⁺ cells and a significant increase in SIINFEKL-H-2K^b cells within the CD11c⁺ population compared with ODA LNPs (Fig. S20).

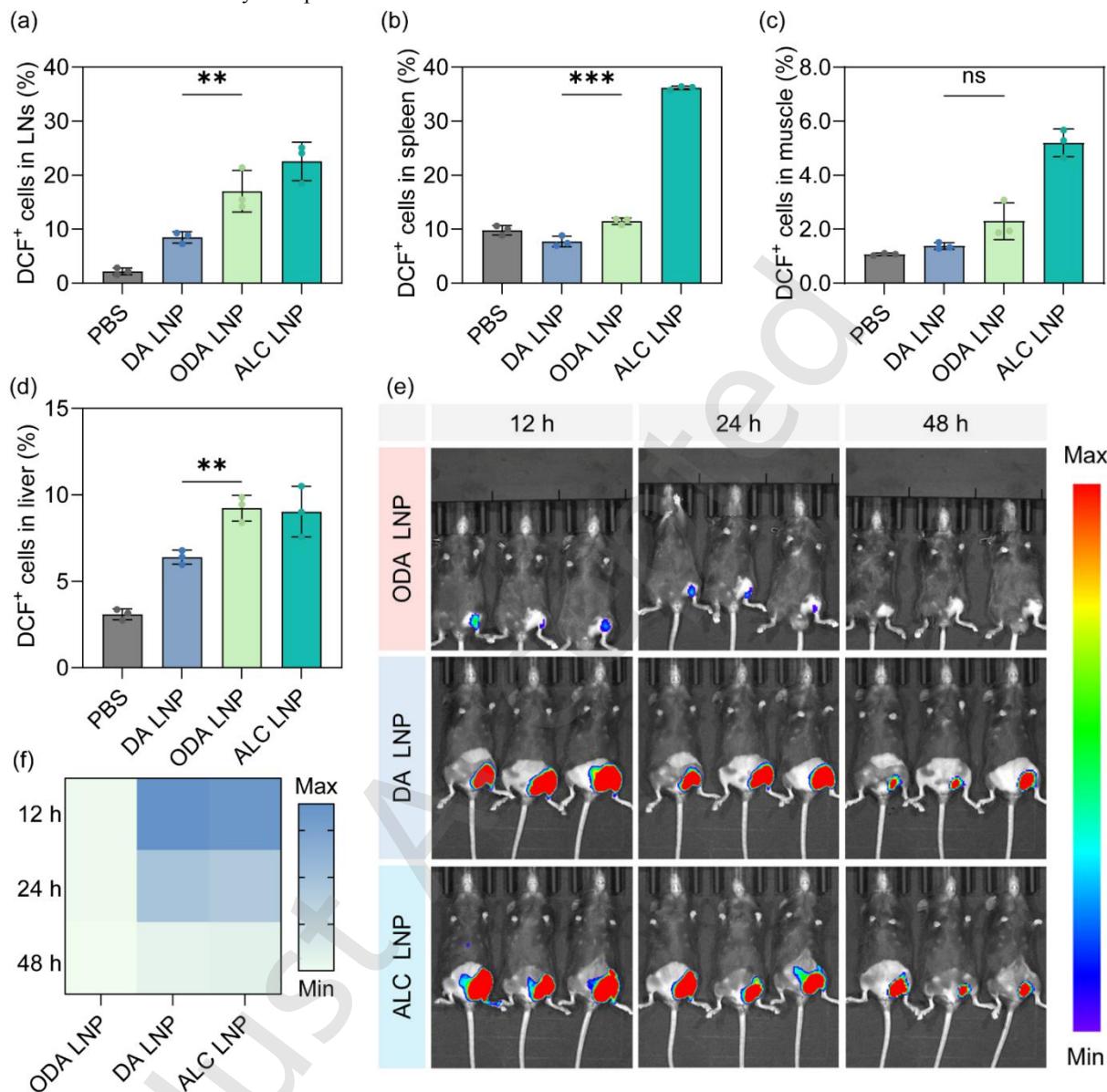


Figure 2 ROS-scavenging capacity and transfection of DA LNPs *in vivo*. (a–d) *In vivo* ROS analysis by flow cytometry of single-cell suspensions from a) lymph nodes, b) spleen, c) muscle, and d) liver collected 12 h post intramuscular injection. DA LNPs-treated tissues showed ROS levels approaching PBS controls, while ALC LNPs and ODA LNPs exhibited elevated ROS ($n = 3$). (e) *In vivo* bioluminescence imaging and (f) corresponding mean bioluminescence signal after intramuscular injection of luciferase mRNA-LNPs. DA LNPs mediated stronger and more sustained signals compared with ODA LNPs, and comparable to ALC LNPs. ($n = 3$). Data are presented as mean $\pm$ SD. Statistical significance was carried out by one-way ANOVA (ns: not significant, ** $p < 0.01$, *** $p < 0.001$).

2.4 Cellular Uptake and Biodistribution

The efficacy of mRNA delivery is fundamentally dependent on efficient cellular internalization and subsequent endosomal escape. To evaluate the uptake efficiency of DA LNPs, we incubated DC2.4 cells with Cy5-labeled mRNA formulations for

6 hours. Flow cytometric analysis revealed that both ODA and DA LNPs achieved high internalization efficiency, notably, DA LNPs yielded a Cy5⁺ cells ratio of approximately 90%, slightly higher than that of the ODA group (Fig. 3(a)).

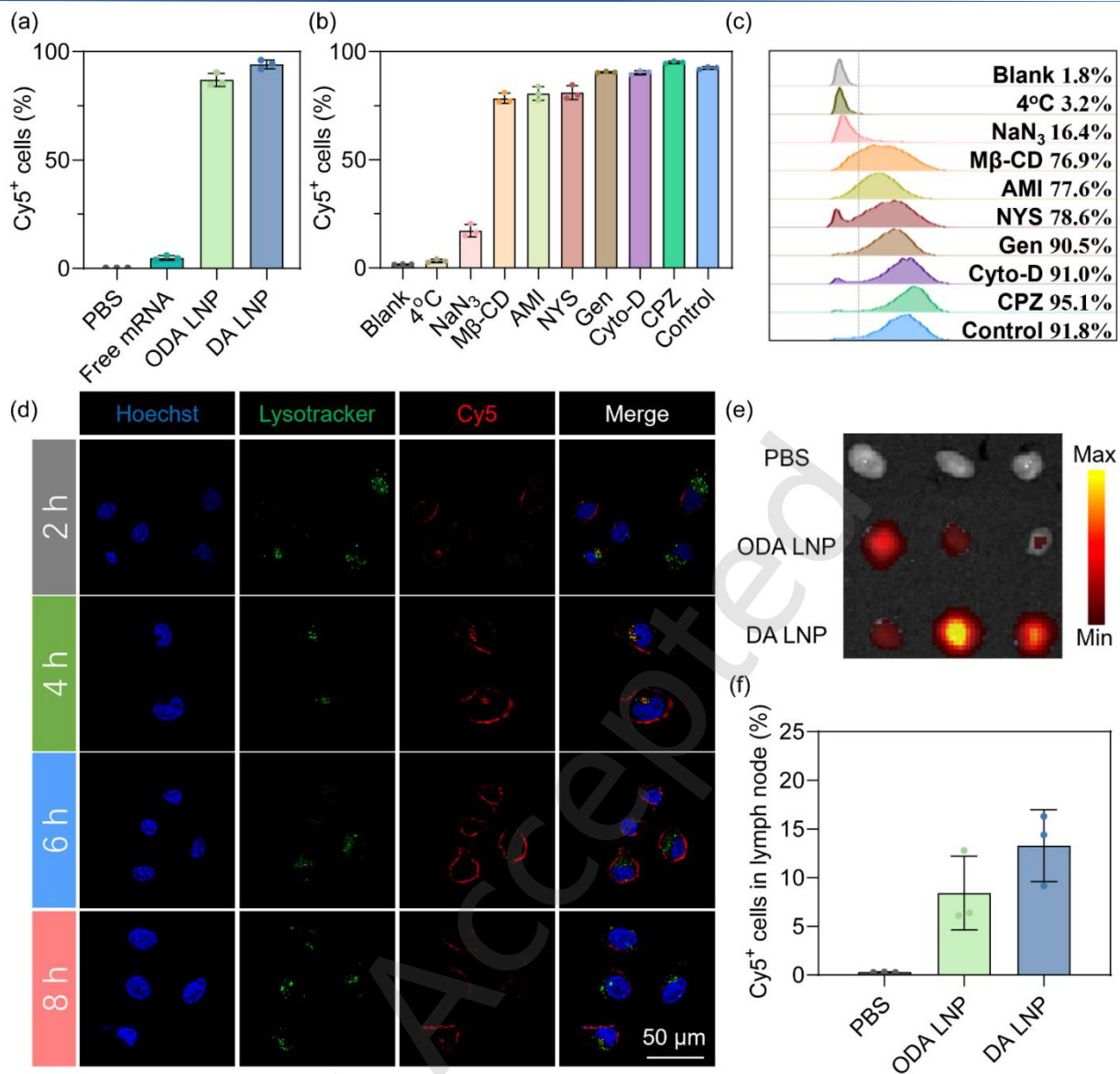


Figure 3 Cellular uptake, endocytic pathways and lymph node distribution of DA LNPs. (a) Uptake of Cy5-labeled mRNA in DC2.4 cells quantified by flow cytometry. DA LNPs yielded a Cy5⁺ cells ratio of approximately 90% ($n = 3$). (b) Percentage of Cy5⁺ DC2.4 cells and (c) Representative FCM results of DC2.4 cells treated with various pharmacological inhibitors ($n = 3$). (d) CLSM images showing intracellular distribution of Cy5-mRNA (red) and colocalization with Lysotracker (green); DA LNPs exhibited time-dependent endosomal escape with reduced colocalization after 8 h. (e) Ex vivo fluorescence imaging and (f) Flow cytometry quantification of Cy5⁺ cells in lymph nodes; DA LNPs increased lymph node delivery compared with ODA LNPs ($n = 3$).

To further elucidate the specific endocytic pathways, pharmacological inhibition assays were performed using methyl- β -cyclodextrin (M β -CD), amiloride (AMI), nystatin (NYS), genistein (Gen), cytochalasin D (Cyto-D), and chlorpromazine (CPZ). Cellular uptake was significantly suppressed at 4°C and upon sodium azide (NaN₃) treatment, confirming that the internalization of DA LNPs is a predominantly energy-dependent active process (Figs. 3(b) and 3(c)). Among the pathway-related inhibitors, M β -CD, AMI, and NYS produced the largest reductions in Cy5-positive cells, suggesting contributions from cholesterol-dependent membrane domains and macropinocytosis to DA LNP uptake. The endosomal escape capability was further investigated via confocal laser scanning microscopy (CLSM). In DC2.4 cells treated with Cy5-mRNA loaded DA LNPs, we observed a progressive cytoplasmic distribution of Cy5 signals. By 8 hours post-incubation, a distinct spatial separation (de-colocalization) between the Cy5 signals and lysosomal compartments was evident (Fig. 3(d)). In representative regions of interest, the

Pearson correlation coefficient between Cy5-mRNA and Lysotracker fluorescence decreased from 2 to 8 h (Fig. S21 in the ESM), further supporting time-dependent endosomal escape.

Efficient accumulation within the lymphatic system is crucial for initiating potent immune responses. Following intramuscular administration, the biodistribution profiles of Cy5-labeled LNPs were evaluated. Ex vivo imaging of draining lymph nodes (dLNs) harvested 12 hours post-injection revealed higher fluorescence intensity in the DA LNP group compared to the ODA group (Fig. 3(e)). Flow cytometric analysis of lymph node cell suspensions showed a substantially higher ratio of Cy5⁺ cells in the DA LNPs-treated mice (Fig. 3(f)). This improved lymphoid targeting likely contributes to more efficient antigen presentation and subsequent immune activation. Consistently, *ex vivo* bioluminescence imaging showed higher luciferase expression in inguinal lymph nodes 12 h after intramuscular administration of DA LNPs than ODA LNPs (Fig. S22 in the ESM), confirming functional mRNA delivery to dLNs.

2.5 Antitumor Efficacy and Immune Responses

We evaluated the prophylactic efficacy of OVA mRNA-loaded DA LNPs in a B16-OVA melanoma model (Fig. 4(a)). DA LNPs suppressed tumor growth compared with PBS and ODA LNPs and were comparable to ALC LNPs (Fig. 4(b); Fig. S23 in the ESM), while body weights remained stable across all groups (Fig. S24 in the ESM). Representative H&E-stained tumor sections from all four groups are shown in Fig. S25 in the ESM.

Current melanoma immunotherapies commonly aim to enhance T-cell activation and function and to promote T-cell infiltration into the tumor microenvironment[42, 43]. Tumor analysis showed that DA LNP vaccination increased the percentage of CD8⁺ cells among CD3⁺ T cells and the percentage of IFN- γ ⁺ cells among CD8⁺ T cells compared

with PBS and ODA LNP vaccination; both responses were comparable to those induced by ALC LNPs (Figs. 4(c), 4(d), and 4(e); Fig. S26 in the ESM). DA and ALC LNP vaccination also increased the percentage of CD86⁺ F4/80⁺ cells within the CD11b⁺ population (Fig. 4(f); Fig. S27 in the ESM), consistent with enrichment of a CD86 expressing macrophage-like phenotype.

Accordingly, we further examined the systemic and intratumoral T cell responses induced by the different LNP formulations. Splenic CD44⁺ CD62L⁺ fractions within both CD4⁺ and CD8⁺ T cell populations were higher after DA LNP vaccination than after PBS or ODA LNP vaccination and comparable to ALC LNPs (Figs. 4(g) and 4(h); Fig. S28 in the ESM), indicating an increased central-memory T-cell phenotype.

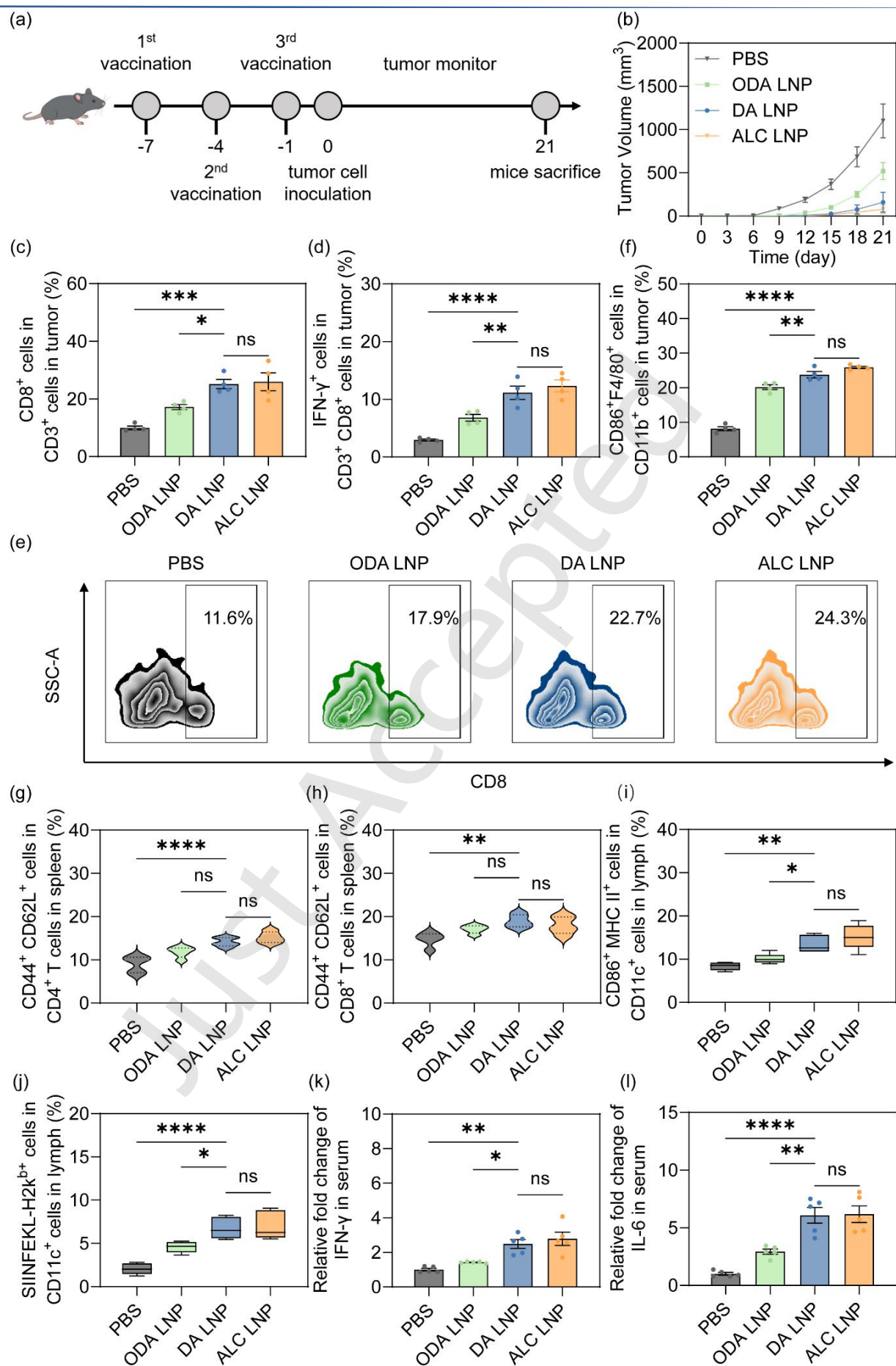


Figure 4 Prophylactic antitumor efficacy and immune responses of OVA mRNA-loaded LNPs in the B16-OVA melanoma model. (a) Vaccination and tumor-challenge schedule. (b) Tumor growth curves. (c) Percentage of CD8⁺ cells among CD3⁺ tumor-infiltrating T cells. (d) Percentage of IFN- γ ⁺ cells

among CD8⁺ tumor-infiltrating T cells. (e) Representative flow-cytometry plots of tumor-infiltrating CD8⁺ T cells. (f) Percentage of CD86⁺ F4/80⁺ cells among CD11b⁺ cells. (g, h) Percentages of CD44⁺ CD62L⁺ cells among splenic CD4⁺ and CD8⁺ T cells, respectively. (i) Percentage of CD86⁺ MHC II⁺ cells and (j) percentage of SIINFEKL-H-2K^b cells among CD11c⁺ cells in draining lymph nodes. Relative fold changes in serum (k) IFN- γ and (l) IL-6. Data are presented as mean $\pm$ SD or as plotted. For the tumor growth, splenic T cell, draining lymph node, and serum analyses, n = 5; for the tumor tissue flow cytometry analyses in panels (c), (d), and (f), n = 4. Statistical significance was determined by one-way ANOVA (ns: no significant, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

Beyond antigen uptake, DC maturation is required for efficient antigen presentation and subsequent T-cell priming[44, 45]. We therefore evaluated DC activation and antigen presentation in the draining lymph nodes. In draining lymph nodes, DA LNP vaccination increased the percentages of CD86⁺ MHC II⁺ and SIINFEKL-H-2K^b cells within the

CD11c⁺ population compared with PBS and ODA LNP vaccination, with responses comparable to those induced by ALC LNPs (Figs. 4(i) and 4(j); Fig. S29 in the ESM). Serum IFN- γ and IL-6 levels were also higher in the DA and ALC LNP groups than in the PBS and ODA LNP groups (Figs. 4(k) and 4(l)), indicating vaccine-induced immune activation.

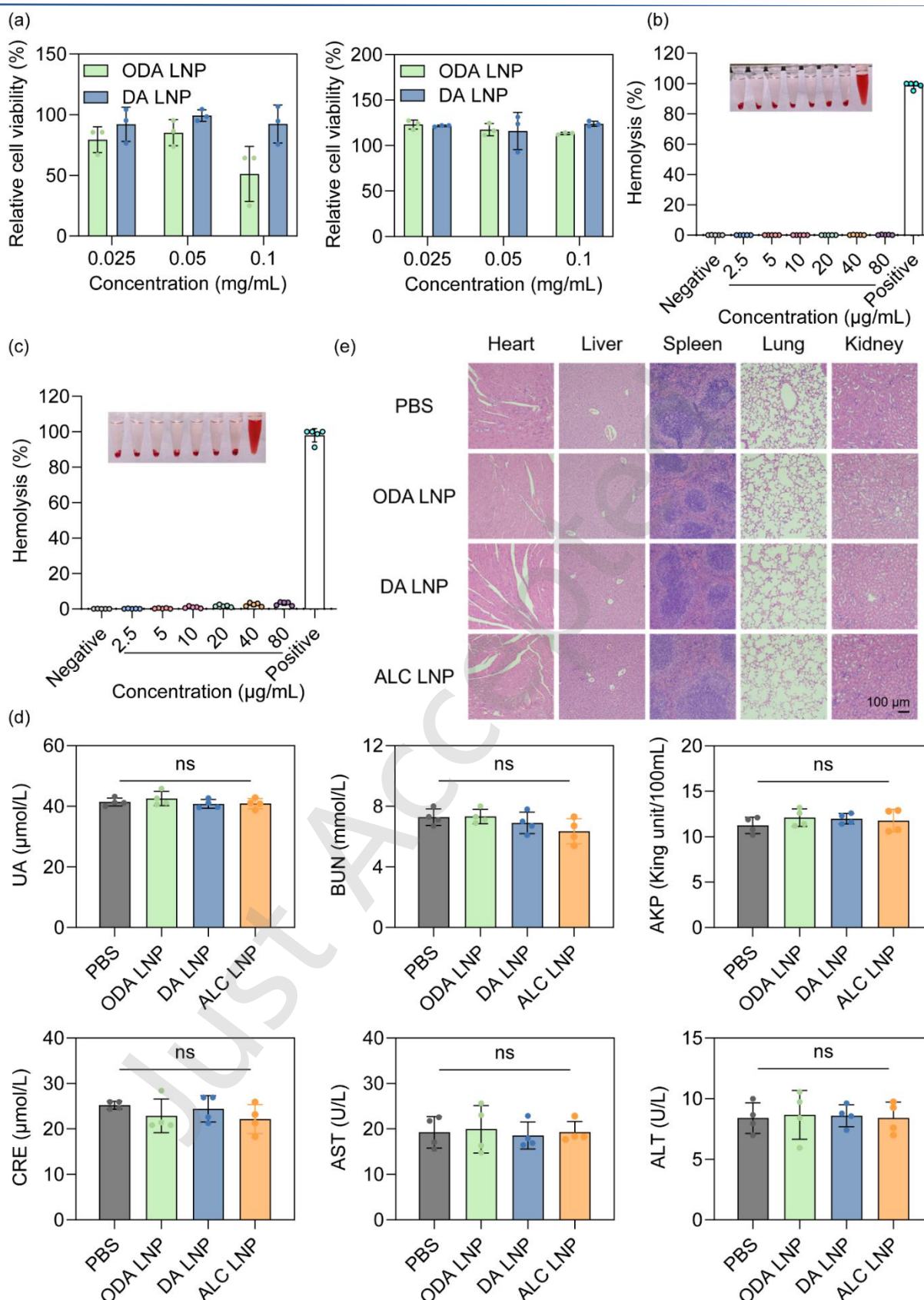


Figure 5 Biosafety evaluation of the LNP formulations. (a) Relative cell viability of HEK293T (left) and DC2.4 cells (right) treated with different concentrations of DA or ODA LNPs, as assessed using the CCK-8 assay. (b) Hemolysis ratio of various concentration of DA LNP and (c) ODA LNP. (d) The blood biochemical analysis of mice. (e) Histological analysis of major organs using H&E staining. Scale bar, 100 μm. Data are presented as mean ± SD. Statistical significance was calculated by one-way ANOVA (ns: no significant).

2.6 Biosafety Evaluation

The translational potential of DA LNPs was assessed through comprehensive safety studies. CCK-8 assays showed no significant reduction in the cell viability of HEK293T or DC2.4

cells following treatment with the tested concentrations of DA LNPs (Fig. 5(a)). Hemolysis assays demonstrated that both ODA LNPs and DA LNPs caused less than 5% hemolysis at all tested concentrations, indicating excellent blood compatibility

(Fig. 5(b) and 5(c)). *In vivo* safety assessment revealed no significant differences in serum biochemical parameters between DA LNP treated and healthy mice, suggesting absence of hepatotoxicity or nephrotoxicity (Fig. 5(d)). Histopathological examination of major organs showed intact tissue architecture without signs of inflammation or damage, confirming the favorable safety profile of DA LNPs for potential clinical application (Fig. 5(e)).

3 Conclusions

In this work, we report a novel ionizable lipid that effectively leverages the intrinsic antioxidant activity of dopamine. DA LNPs demonstrated excellent ROS scavenging capability, enhanced mRNA-derived protein expression, and improved antigen presentation capacity. In a prophylactic melanoma model, DA LNPs elicited potent antitumor immunity through multiple mechanisms including enhanced lymphoid accumulation, improved T cell memory formation. This study highlights the crucial importance of redox modulation in mRNA vaccine design and provides a feasible strategy for developing high-performance nucleic acid delivery systems. The favorable safety profile and efficacy of DA LNP support further preclinical evaluation of DA LNP.

4 Experimental

4.1. Synthesis of DA lipid

To a solution of 2-hexyldecanoic acid (2 g, 7.8 mmol) in dichloromethane (40 mL) was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 1.53 g, 9.84 mmol). The reaction was stirred at room temperature for 2 hours, then added 4-dimethylaminopyridine (DMAP, 0.18 g, 1.52 mmol) and 6-bromo-1-hexanol (1.84 g, 10.14 mmol) into the system and stirred at room temperature for 24 hours. The mixture was diluted with dichloromethane and washed three times with saturated aqueous sodium bicarbonate solution, then brine, and the organic layer was dried with sodium sulfate. The solvent was removed in vacuo, the crude product was purified by silica gel chromatography packed and eluted in dichloromethane to give 6-bromohexyl 2-hexyldecanoate as a pale-yellow oil.

2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)ethan-1-amine (DA (ac)) was synthesized as reported[46].

To a solution of lipid tail (1.29 g, 3.08 mmol) in anhydrous acetonitrile (10 mL) was added sodium iodide (200 mg, 1.33 mmol) and potassium carbonate (966 mg, 6.99 mmol). Then, DA(ac) (265mg, 1.40 mmol) was added and the mixture was degassed with three freeze-pump-thaw cycles, after that the reaction was heated to a reflux at 90 °C and stirred for 48 hours. The solvent was collected after vacuum filtration and removed via evaporation in vacuo, the crude product was further purified via silica gel chromatography (0-25% ethyl acetate in hexanes), and the fraction containing the product was collected and condensed in vacuo and dried under vacuum to give ((2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)ethyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate), which was then dissolved in TFA/CH₂Cl₂ (1:4) and stirred for 4 hours at room temperature to produce the DA lipid.

4.2 Preparation of LNPs formulation

LNPs were formulated at a 20:1 ratio of total lipid to mRNA. Ionizable lipid, cholesterol, phospholipid, and PEG-lipid were

dissolved in ethanol with a molar ratio of 50/38.5/10/1.5. mRNA was dissolved in a 10mM citrate buffer (pH = 3). Then, the aqueous and ethanol phases were mixed at 3/1 volumetric ratio to produce LNPs. LNPs were dialyzed against PBS for 2 h.

4.3 Particle size and potential determination

The zeta potential and particle size of nanoparticle at the best ratio was measured using the Zetasizer Nano ZS system (Zetasizer Nano ZS-90, Malvern). Samples were diluted 100 times using deionized water, and then transferred to DLS cuvette and zeta cell respectively for data acquisition.

4.4 Gel retardation assay

LNPs were prepared and then mixed with RNA loading buffer solution. Weigh 1 g agarose gel into a flask, add 49 mL Milli-Q water and 1mL 50 × TAE buffer solution, then heat it until a slight boil. Add 5 μL 10000 × SolarRed nucleic acid dye, mix well and pour the mixture into a mold to let it solidify at room temperature. After 15 min, remove the comb and place the agarose gel into an electrophoresis apparatus, carefully adding the test sample into the gel wells. Run at 100 V for 30 minutes and place the gel under an imaging device for observation.

4.5 ROS scavenging capacities assay

For total antioxidant capacity against ROS for DA lipid and ODA lipid, we measured strictly in accordance with the instruction of T-AOC Assay Kit (S0119, Beyotime) based on the ABTS agent.

For antioxidant capacity against ·O²⁻ for DA lipid and ODA lipid, the pyrogallol autoxidation method was employed for the assay. Pyrogallol undergoes autoxidation under weakly alkaline conditions to generate superoxide anions and colored intermediates, where the absorption intensity is proportional to the amount of superoxide anions. The sample to be tested was dispersed in Tris-HCl buffer (pH = 8.0). Subsequently, pyrogallol (dissolved in 1 mM HCl) was added to the mixture to reach a final concentration of 250 μM, followed by incubation at room temperature for 20 min. The absorbance values of the pure pyrogallol solution (A₀) and the mixed solution of pyrogallol and sample (A_s) were measured at 319 nm. The superoxide anion scavenging rate of the sample was calculated according to the following formula: Scavenging rate (%) = ((A₀-A_s)/A₀)×100%.

For antioxidant capacity against DPPH radical for DA lipid, ODA lipid and the corresponding LNPs, the DPPH radical scavenging assay was conducted. Briefly, a 1.5 mM DPPH working solution was prepared. Then, 200 μL of the working solution and 10 μL of the sample to be tested at various concentrations were added to each well of a 96-well plate, followed by incubation at 37 °C for 30 min. The absorbance values of the DPPH-ethanol solution (A_c), the DPPH-sample mixture (A_s), and the sample-ethanol solution (A_b) were measured at 517 nm. The DPPH radical scavenging rate of the sample was calculated according to the following formula: Scavenging rate (%) = [1-(A_s-A_b)/A_c]×100%.

For antioxidant capacity against ROS for DA LNP and ODA LNP *in vitro* and *in vivo*, we measured strictly in accordance with the instruction of Reactive Oxygen Species Assay Kit (S0033S, Beyotime) based on the DCFH-DA probe.

4.6 NAC pretreatment assay

For the cell viability assay, DC2.4 cells were treated with NAC at concentrations of 0, 1, 2, and 5 mM for 24 h. The cells were then washed with PBS, and the CCK-8 assay was

performed in fresh medium according to the manufacturer's instructions.

For NAC pretreatment, DC2.4 cells were incubated with 1 mM NAC for 24 h, washed with PBS, and subsequently transfected with luciferase mRNA or GFP mRNA loaded ALC-0315 and SM102 based LNPs at an mRNA concentration of 1 µg/mL. After 24 h, intracellular ROS was observed using the DCFH-DA probe, luciferase expression was measured and normalized to the total protein content, and GFP-positive cells and mean fluorescence intensity were analyzed by flow cytometry.

4.7 Animals and tumor models

Female C57BL/6 mice (6~7 weeks old) were purchased from Xiamen University Laboratory Animal Center. All animal experimental procedures were conducted according to the protocol approved by Animal Ethics and Use Committee of Xiamen University (XMULAC20230020). For the B16 OVA tumor model, the mice were randomly divided into the following four groups: PBS, ODA LNP, DA LNP, and ALC LNP. The dosage of mOVA was 5 µg, after three doses of PBS, ODA LNP, DA LNP, and ALC LNP administering, the mice were injected subcutaneously in the right dorsal flank region with 100 µL of cell suspension containing B16-OVA cells (1×10^6). The tumor growth profiles and weight changes were monitored every 3 days and the volume was calculated as (tumor length) $\times$ (tumor width)²/2.

4.8 Statistical analyses

Unless otherwise stated, data are presented as mean $\pm$ SD. The definition of n is provided in each figure legend. All statistical analyses were conducted using GraphPad Prism. Significance was determined using one-way ANOVAs with multiple comparisons tests. The significant levels are shown as ns: not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 as indicated.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and/or the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution statement

G. H. F.: Writing – original draft, Investigation, Data curation, Writing – review & editing, Methodology, Formal analysis, Conceptualization. Z. Y. T.: Methodology, Investigation, Formal analysis. H. M. F.: Methodology, Investigation, Data curation. R. Y. W.: Investigation, Methodology, Data curation. Y. M. X.: Methodology, Investigation, Data curation. H. X. L.: Investigation, Methodology, Data curation. L. W. X.:

Methodology, Investigation. Z. W. S.: Methodology, Data curation. C. Z.: Methodology, Investigation. K. H.: Writing – original draft, Project administration, Writing – review & editing, Supervision, Conceptualization. H. Y. T.: Writing – review & editing, Project administration, Conceptualization, Writing – original draft, Funding acquisition.

Informed consent

Not applicable.

Ethics statement

The animal experiments were carried out following the guidelines of the Xiamen University Institutional Committee for the Care and Use of Laboratory Animals and were approved by the Committee on Ethical Use of Animals of Xiamen University (Ethical code: XMULAC20230020).

Use of AI statement

None.

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Electronic Supplementary Material

Design of lipid nanoparticles with potent reactive oxygen species scavenging capacity for efficient mRNA delivery

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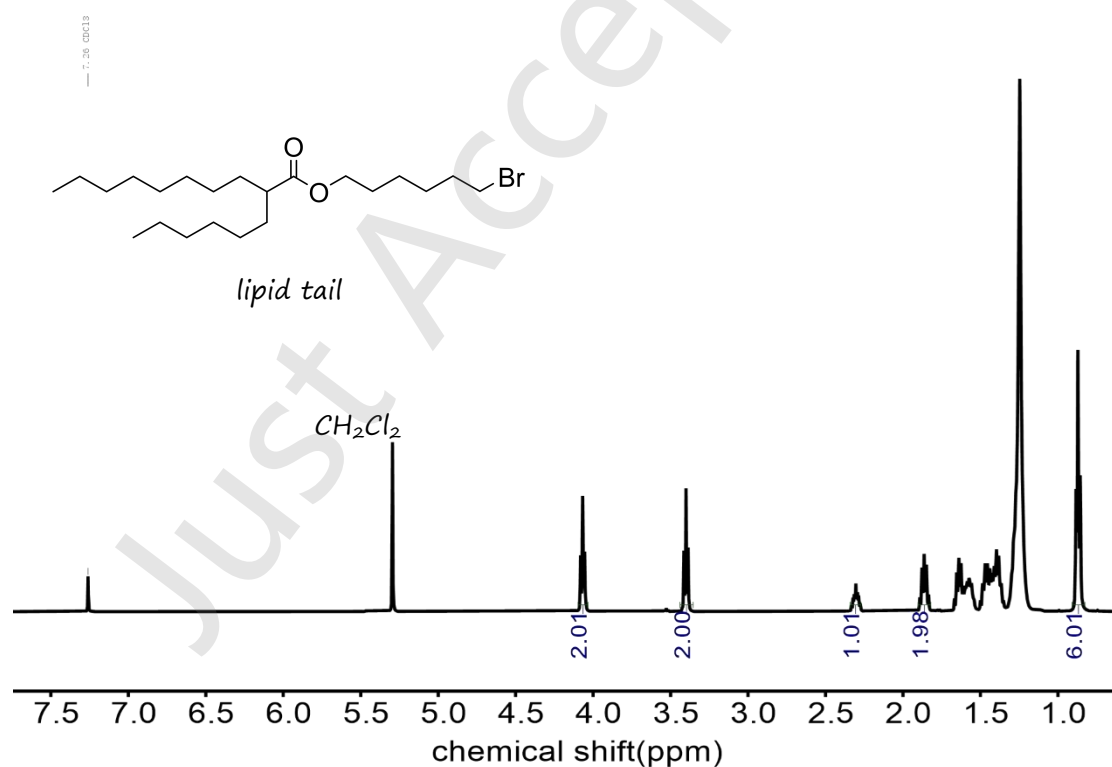


Fig. S1. ¹H NMR spectrum of 6-bromohexyl 2-hexyldecanoate (lipid tail).

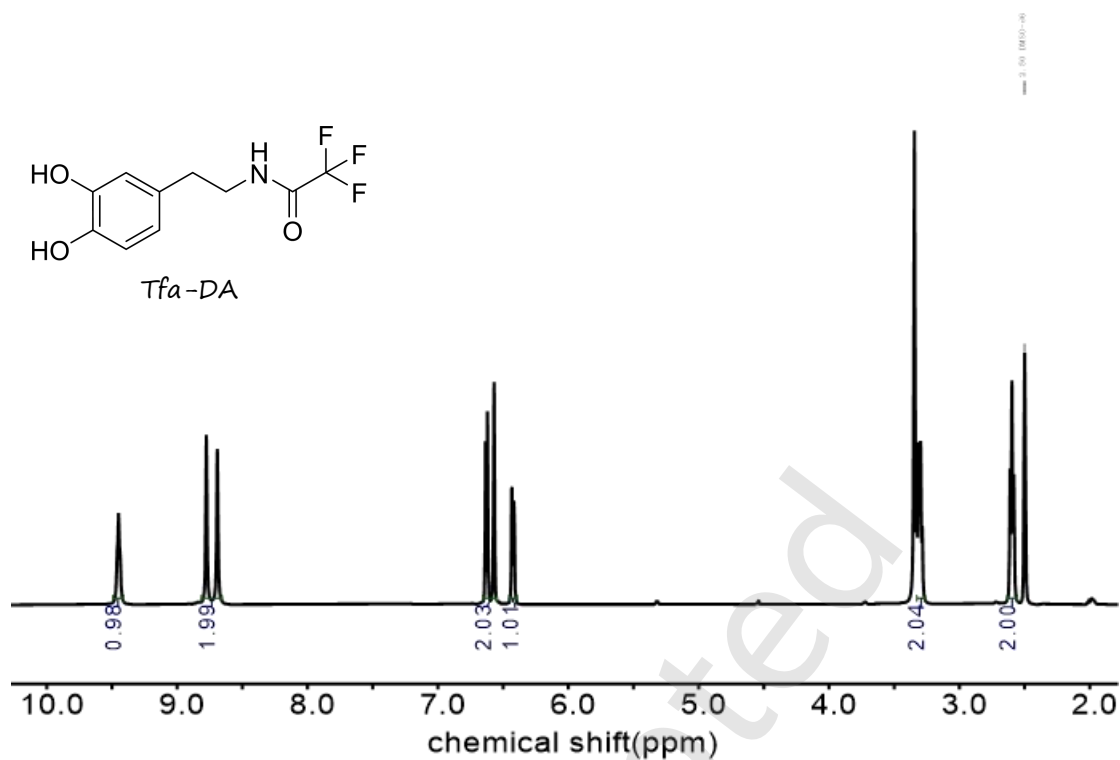


Fig. S2. ¹H NMR spectrum of N-(3,4-dihydroxyphenethyl)-2,2,2-trifluoroacetamide (Tfa-DA).

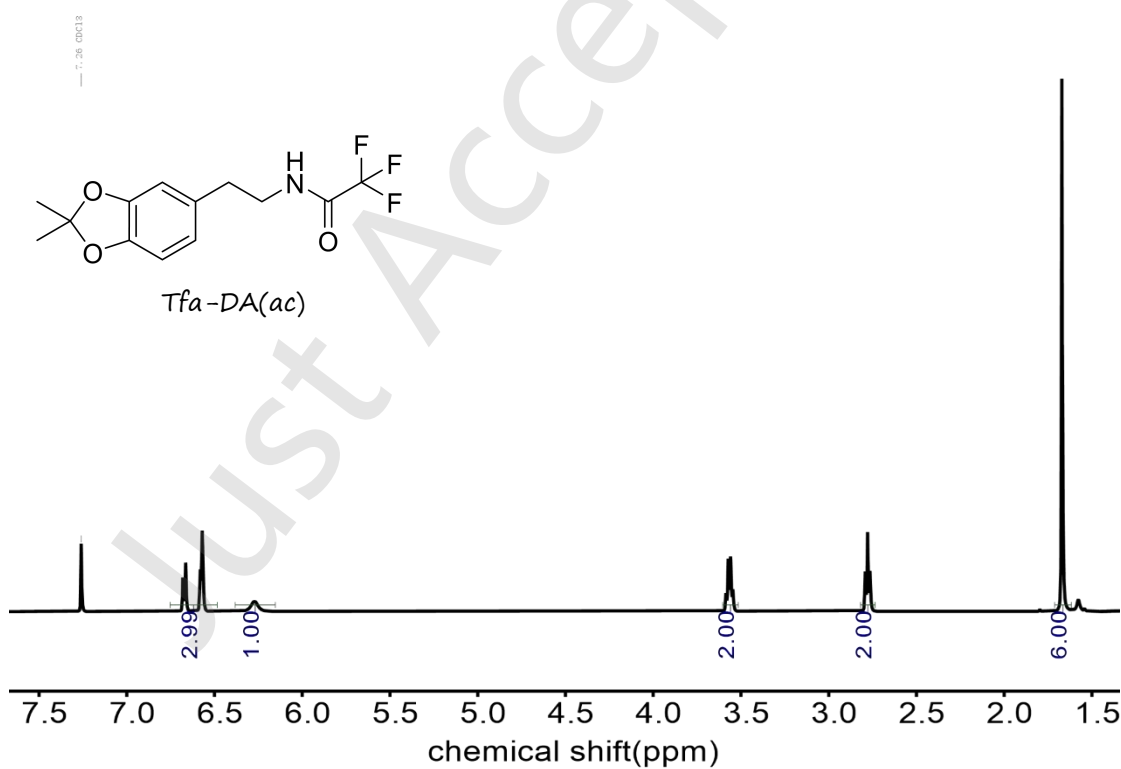


Fig. S3. ¹H NMR spectrum of N-(2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)ethyl)-2,2,2-trifluoroacetamide (Tfa-DA(ac)).

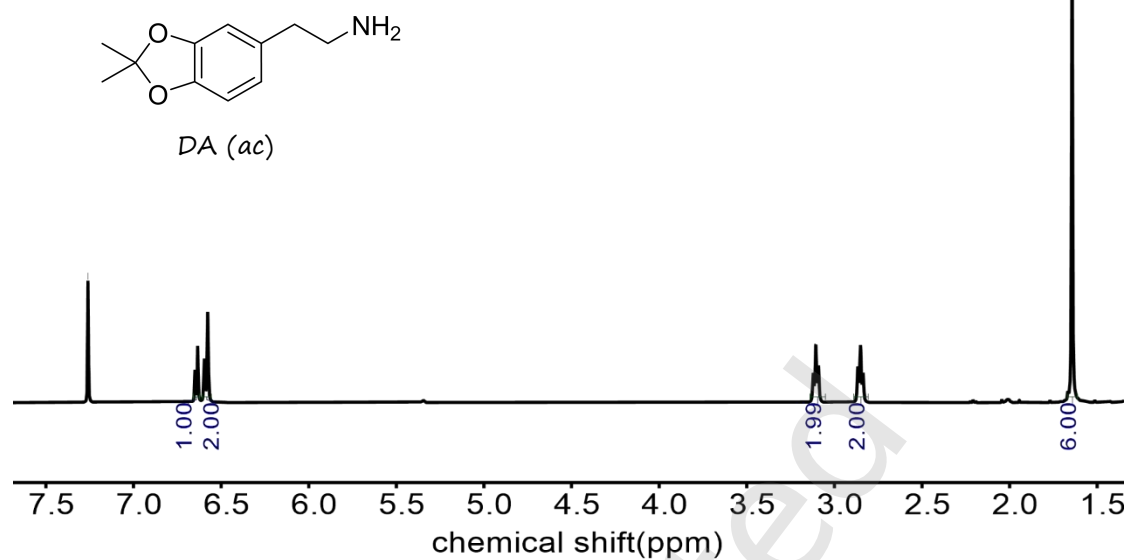


Fig. S4. ¹H NMR spectrum of 2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)ethan-1-amine (DA(ac)).

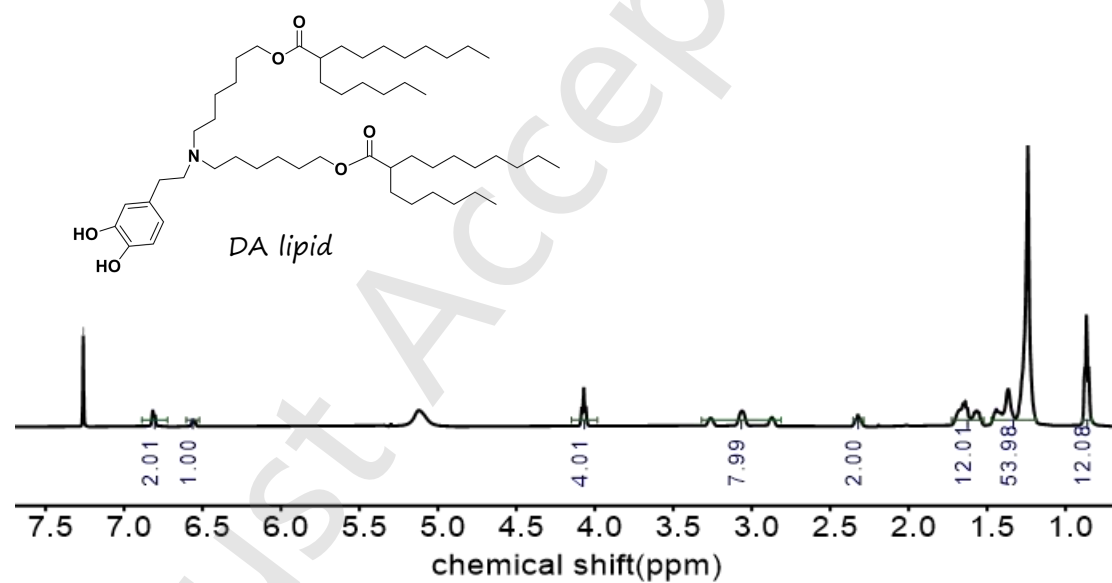


Fig. S5. ¹H NMR spectrum of ((3,4-dihydroxyphenethyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate) (DA lipid).

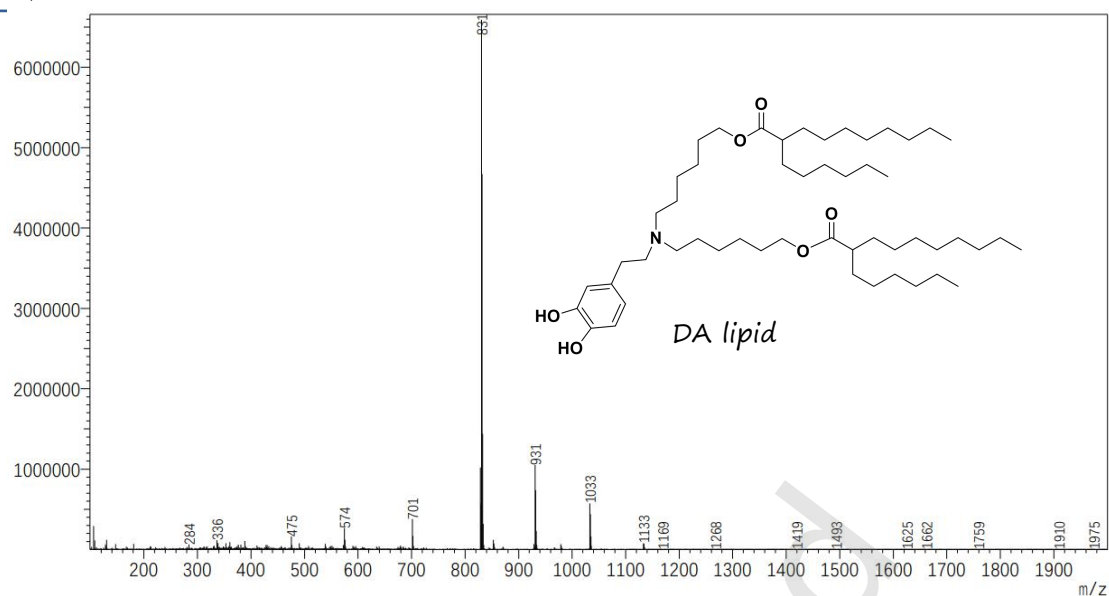


Fig. S6. Mass spectrum of ((3,4-dihydroxyphenethyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate) (DA lipid).

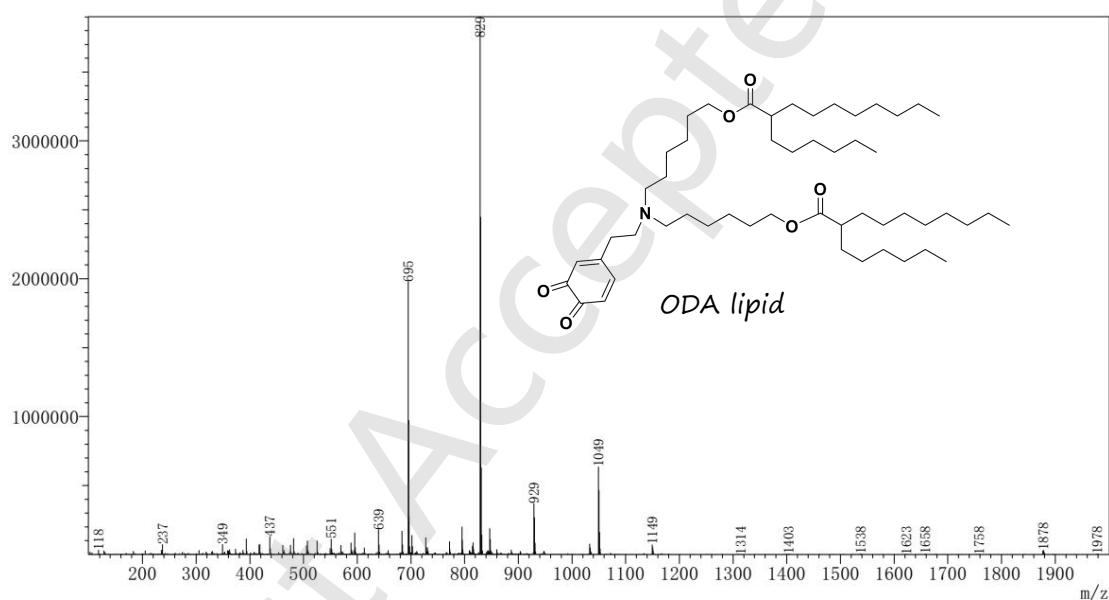


Fig. S7. Mass spectrum of ((2-(3,4-dioxocyclohexa-1,5-dien-1-yl)ethyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ODA lipid).

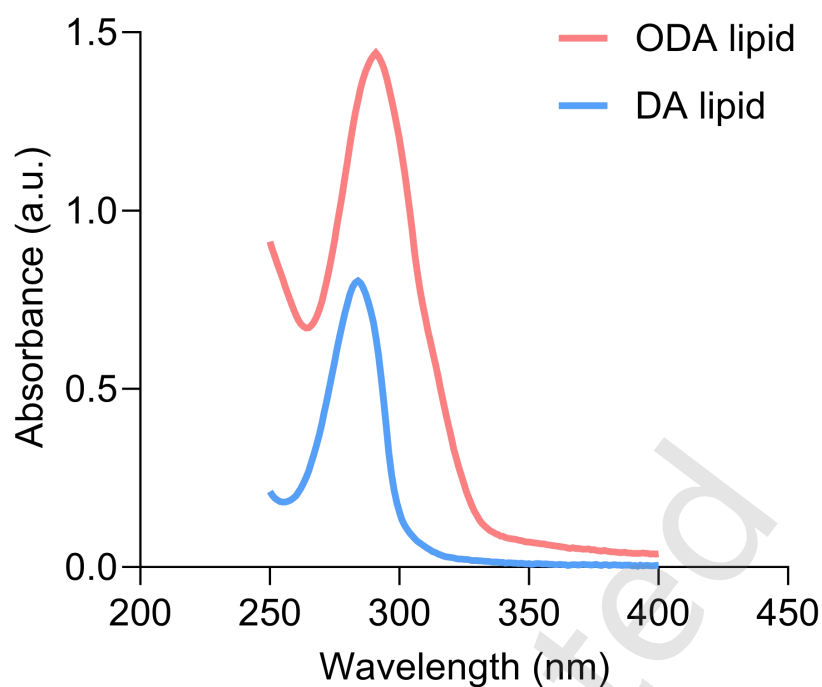


Fig. S8. Ultraviolet-visible spectrum of DA lipid and ODA lipid.

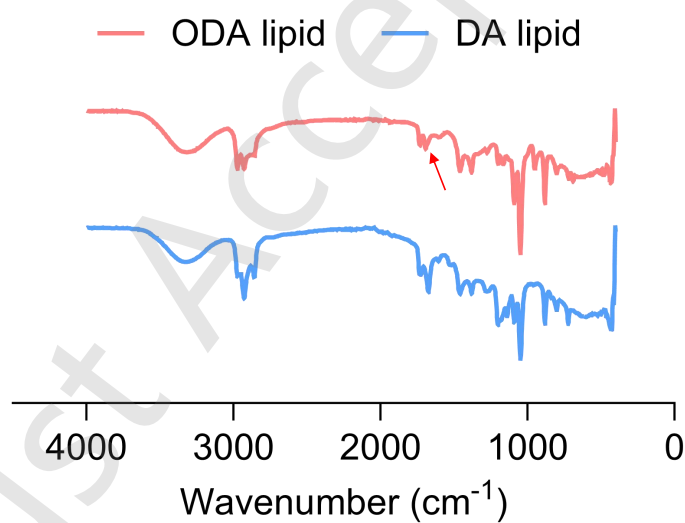


Fig. S9. Infrared spectrum of DA lipid and ODA lipid.

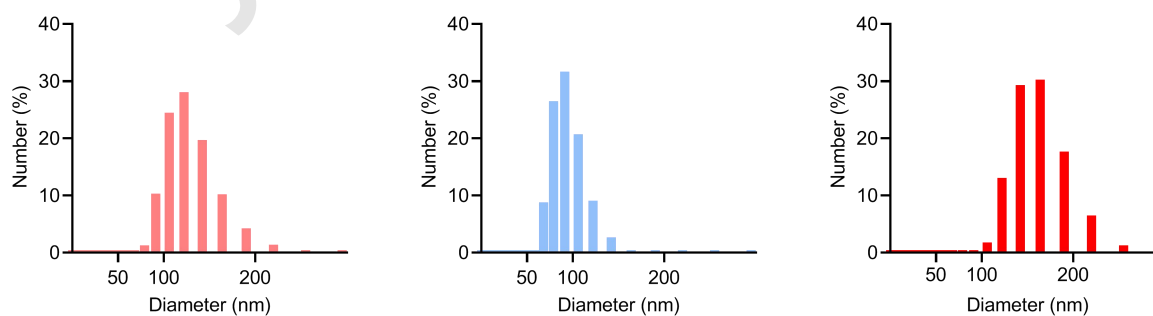


Fig. S10. Hydrodynamic diameters of ODA LNPs (left), DA LNPs (middle) and ALC LNPs (right) determined by DLS.

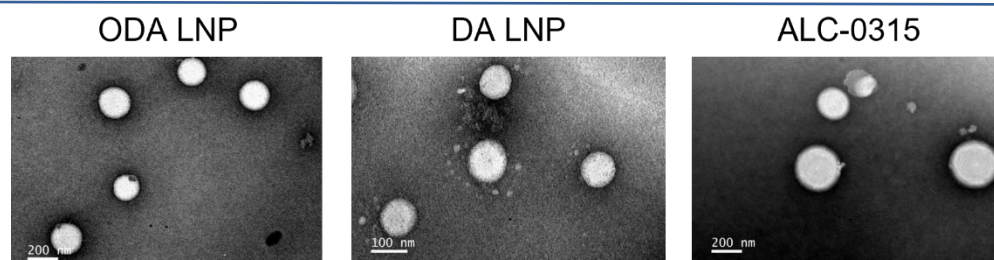


Fig. S11. TEM images of ODA LNPs, DA LNPs and ALC LNPs.

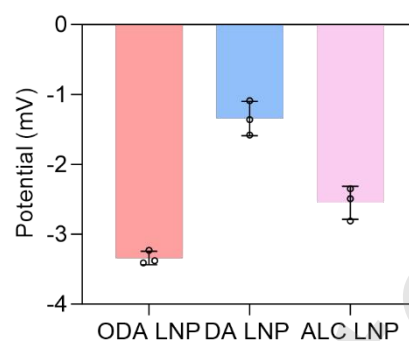


Fig. S12. Zeta potential measurements of LNP formulations; DA LNPs showed a zeta potential closer to neutrality ($n = 3$).

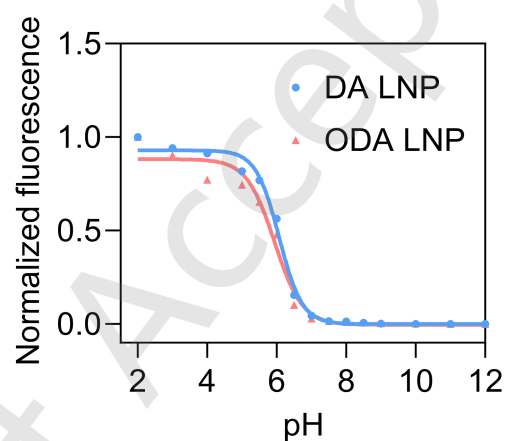


Fig. S13. Apparent pK_a values obtained from TNS fluorescence titration. Both DA and ODA LNPs exhibited values in the optimal range.

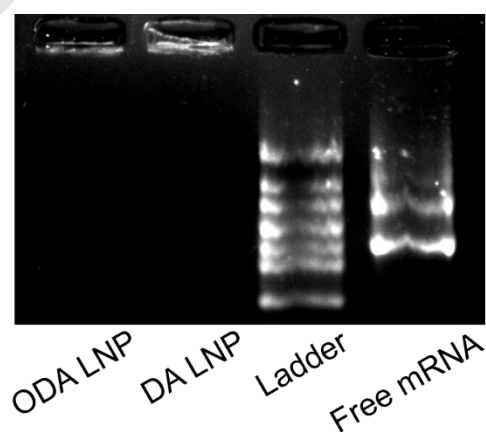


Fig. S14. Agarose gel electrophoresis confirming efficient mRNA encapsulation with negligible leakage.

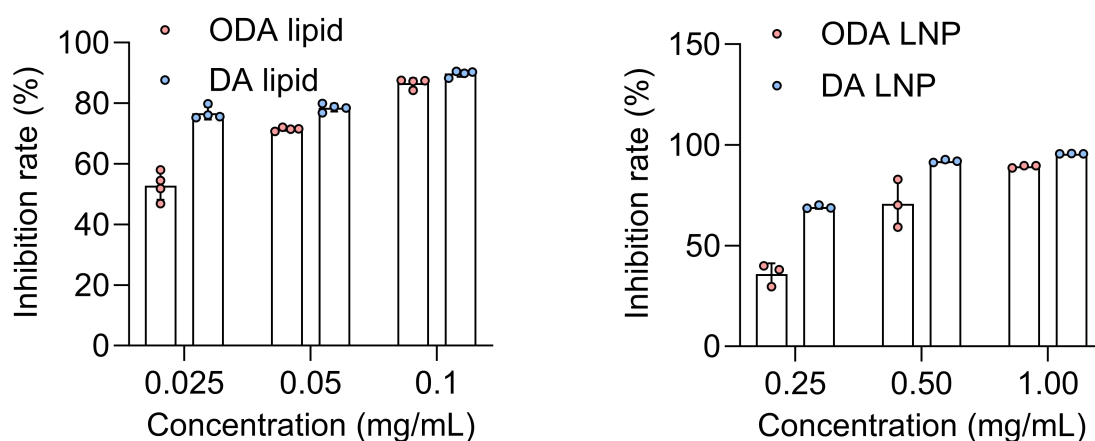


Fig. S15. Results of the DPPH free radical inhibition capability of ODA lipid, DA lipid (left) and the corresponding LNPs (right).

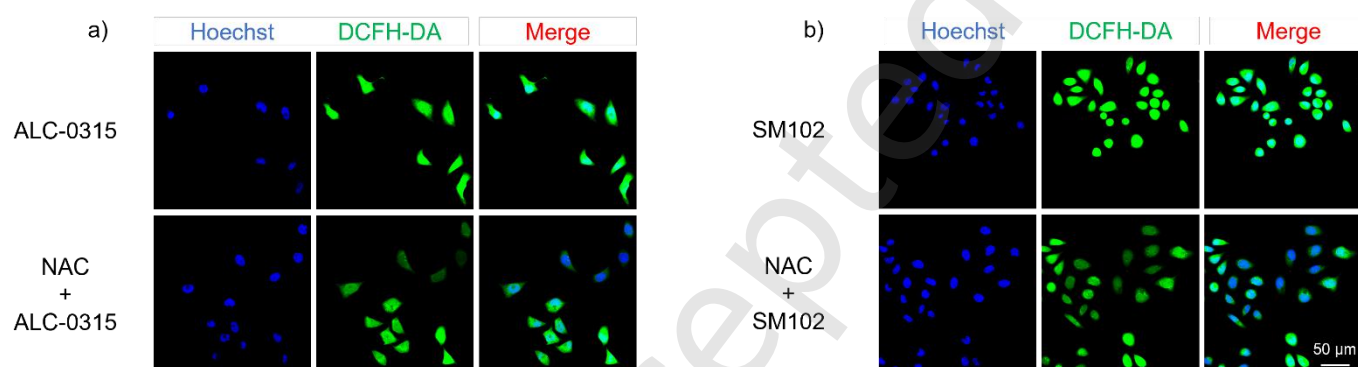


Fig. S16. Representative DCFH-DA fluorescence images of DC2.4 cells transfected with ALC-0315 or SM102 based mRNA-LNPs with or without NAC pretreatment.

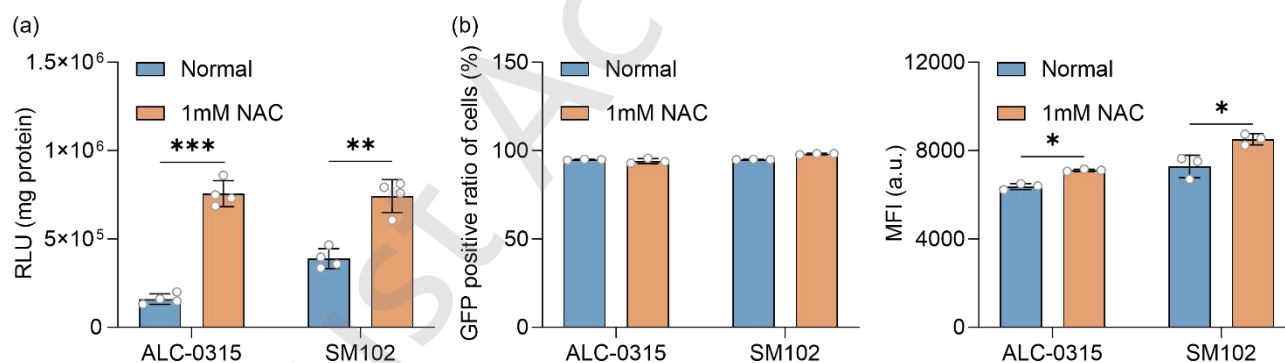


Fig. S17. Effects of NAC pretreatment on mRNA-derived reporter expression in DC2.4 cells.

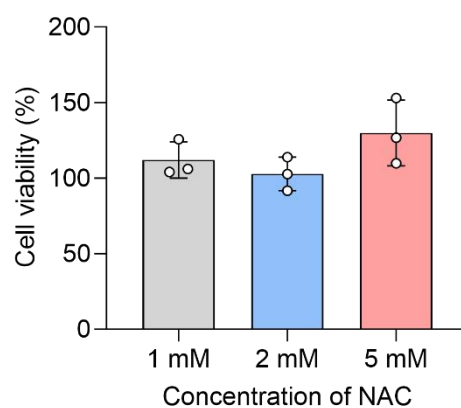


Fig. S18. Relative cell viability of DC2.4 cells after exposure to NAC.

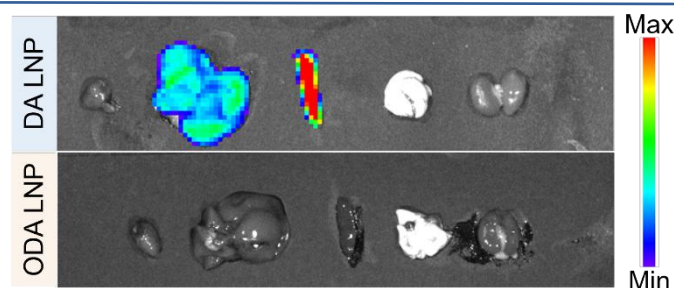


Fig. S19. IVIS luminescence imaging of organs treated with DA LNPs and ODA LNPs containing luciferase mRNA.

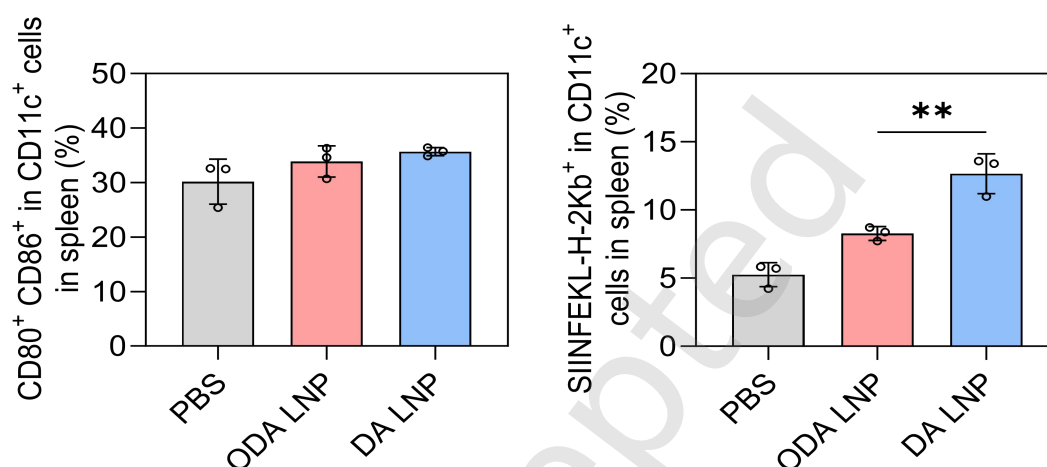


Fig. S20. Flow cytometry analysis of CD80⁺ CD86⁺ and SIINFEKL-H-2Kb⁺ cells in CD11c⁺ dendritic cells after OVA mRNA delivery; DA LNPs induced stronger antigen presentation compared with ODA LNPs (n = 3).

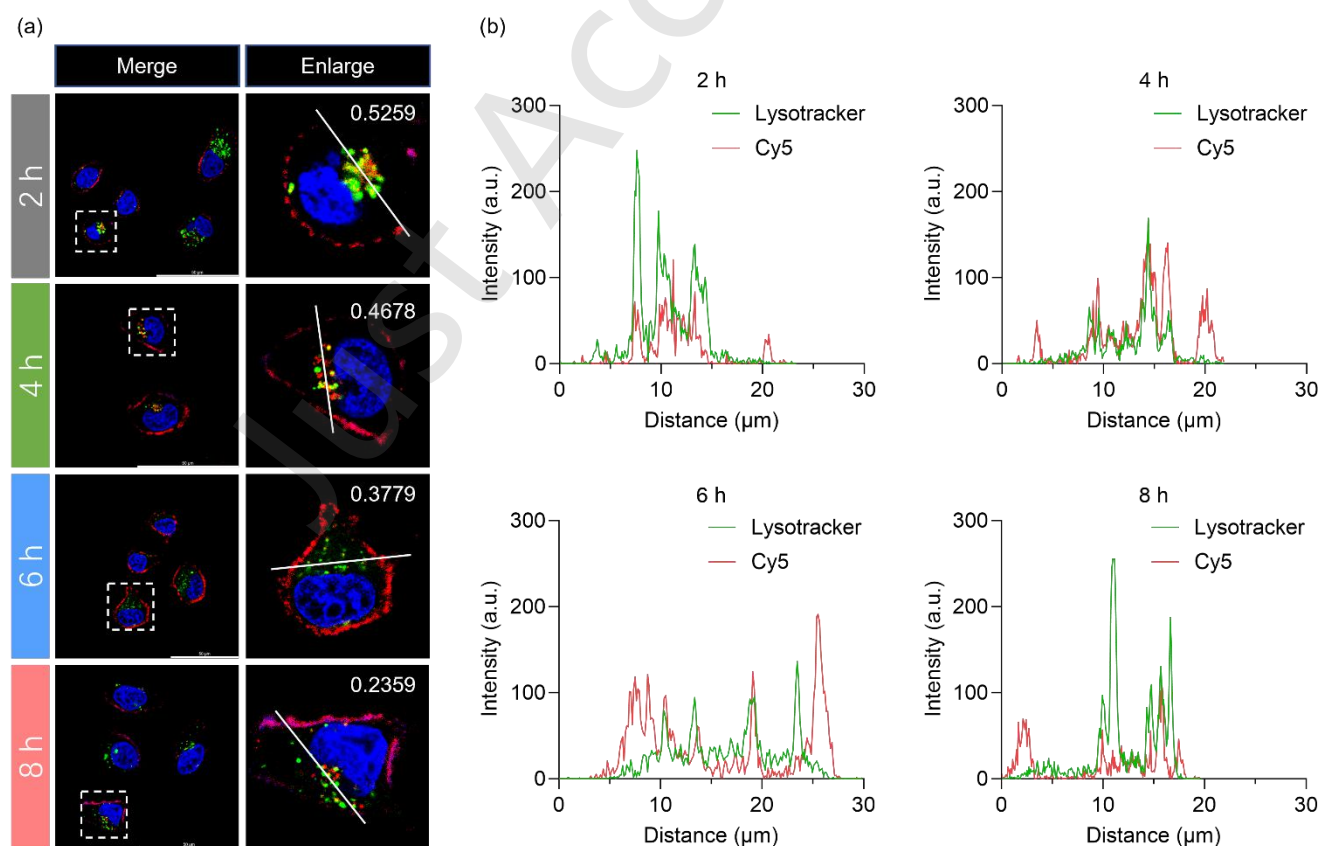


Fig. S21. Representative colocalization analysis of Cy5-labeled mRNA delivered by DA LNPs in DC2.4 cells. Numbers shown in the enlarged images indicate the Pearson correlation coefficients calculated for the displayed regions of interest. Fluorescence intensity profiles of Lysotracker and Cy5-labeled mRNA along the indicated lines are shown at 2, 4, 6, and 8 h.

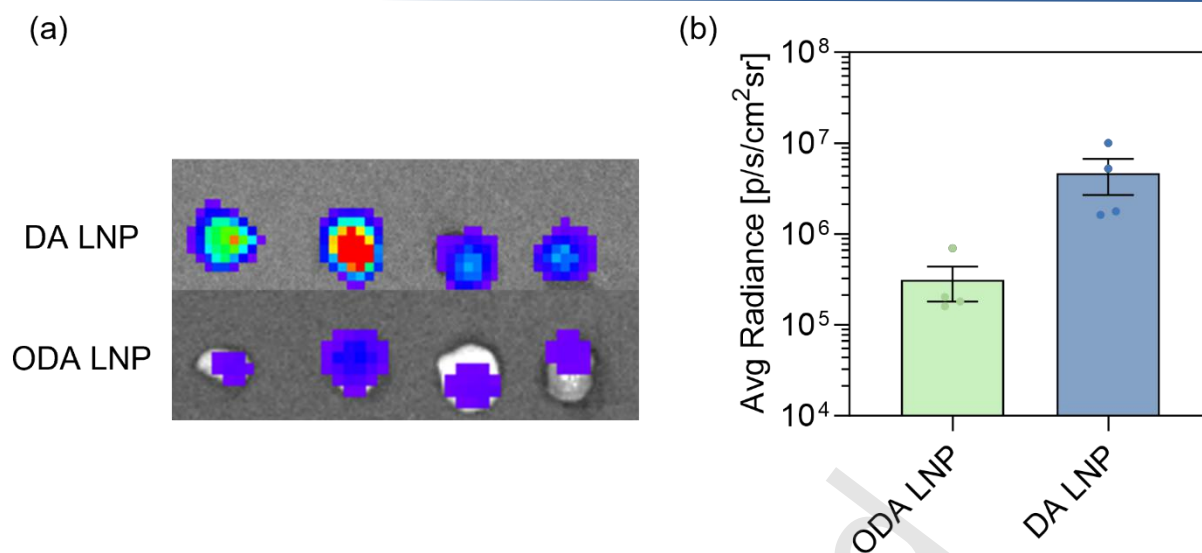


Fig. S22. IVIS luminescence imaging and the average radiance of lymph node after intramuscular administration of luciferase mRNA-loaded LNPs.

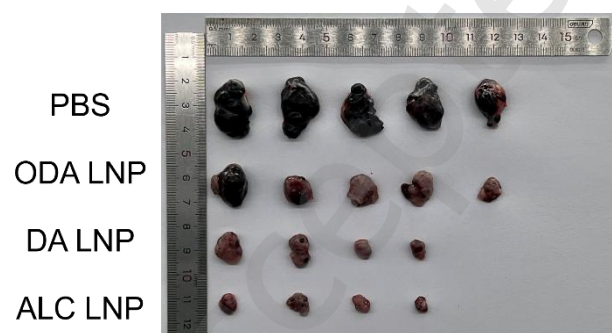


Fig. S23. Representative photographs of excised tumors collected on day 21.

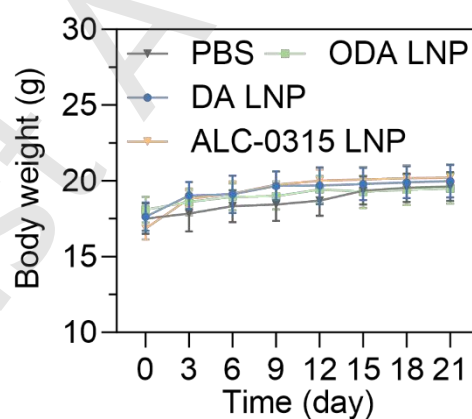


Fig. S24. Body-weight changes of mice in the PBS, ODA LNP, DA LNP, and ALC LNP groups during the B16-OVA prophylactic experiment.

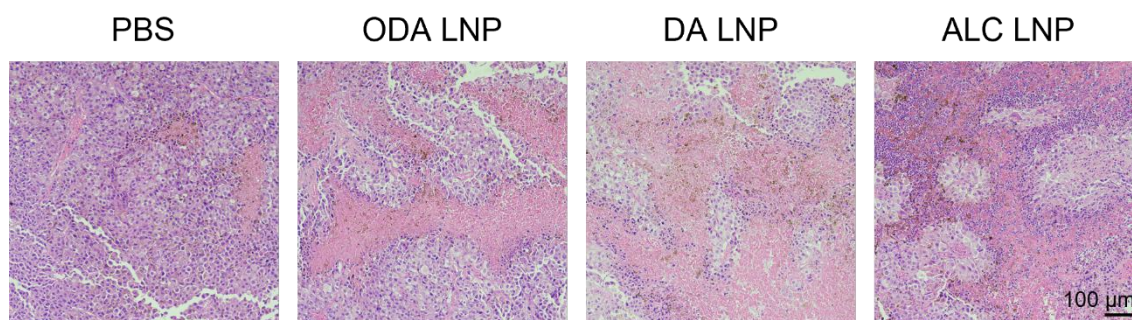


Fig. S25. Representative H&E-stained tumor sections collected on day 21 from mice in the PBS, ODA LNP, DA LNP, and ALC LNP groups. Scale bar, 100 μ m.

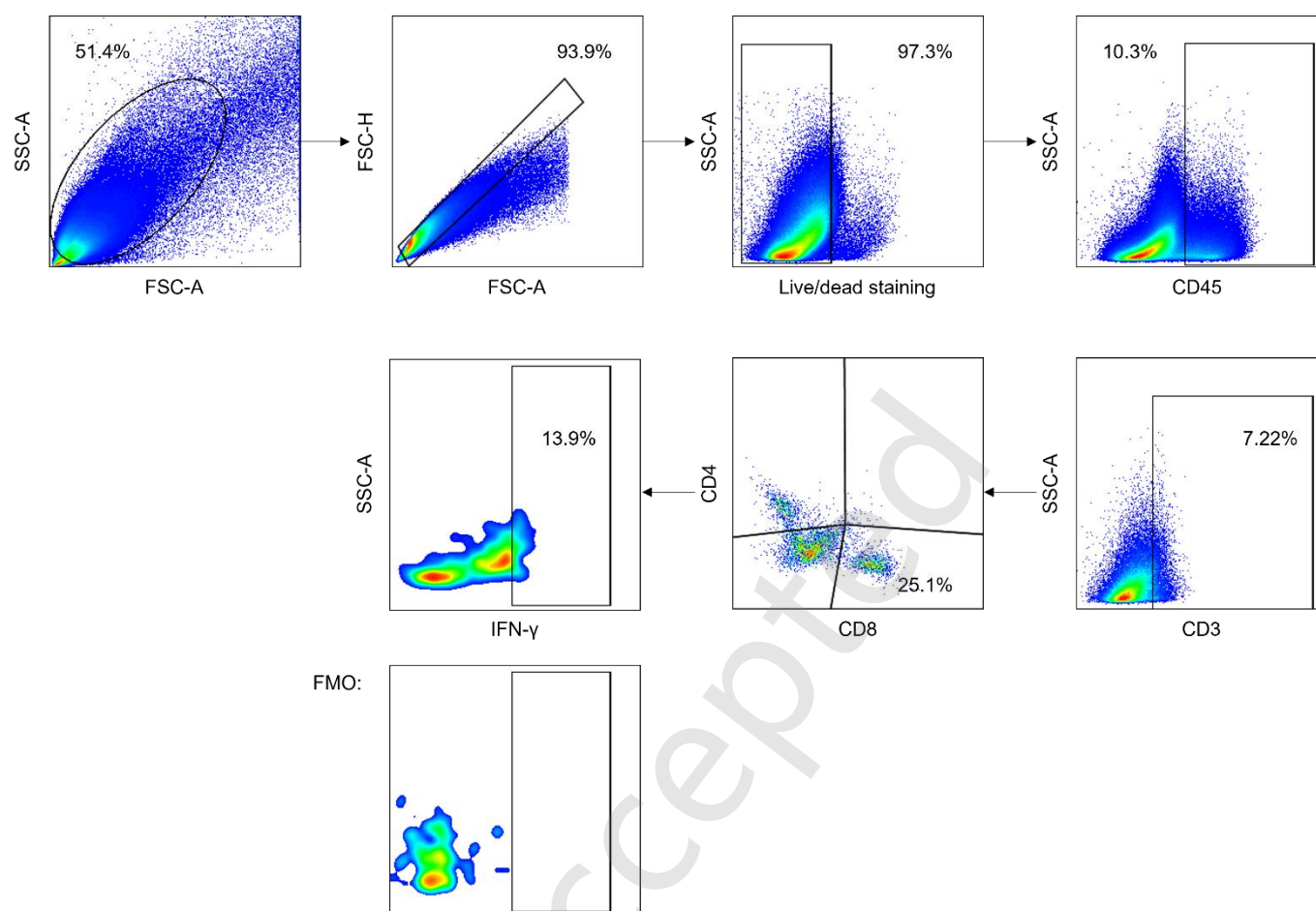


Fig. S26. FCM gating strategy and IFN- γ FMO control for the analysis of IFN- γ ⁺ cells within tumor-infiltrating CD8⁺ T cells in the prophylactic B16-OVA model.

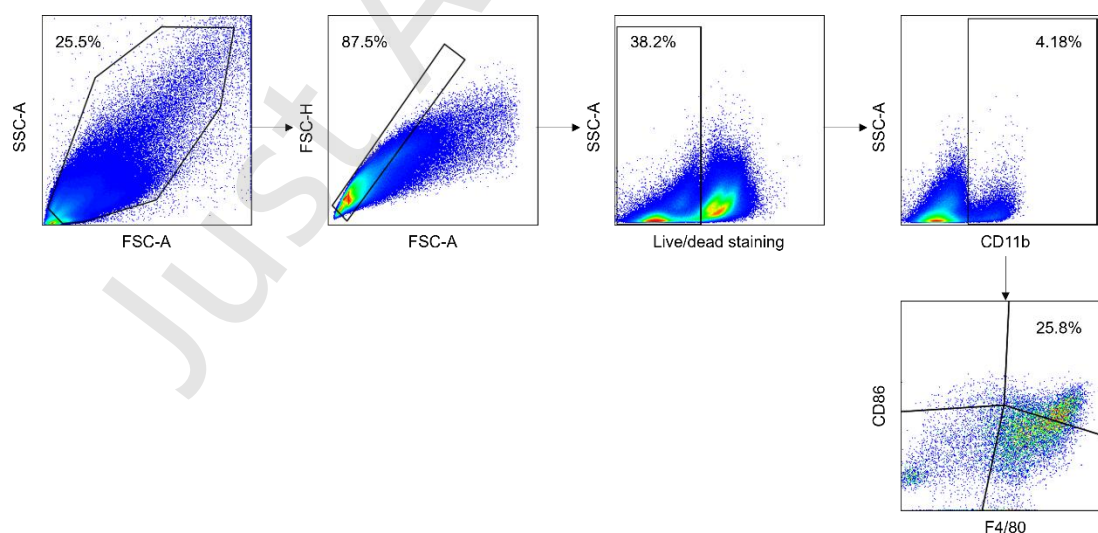


Fig. S27. FCM gating strategy for the analysis of CD86⁺ F4/80⁺ cells within tumor-infiltrating CD11b⁺ cells in the prophylactic B16-OVA model.

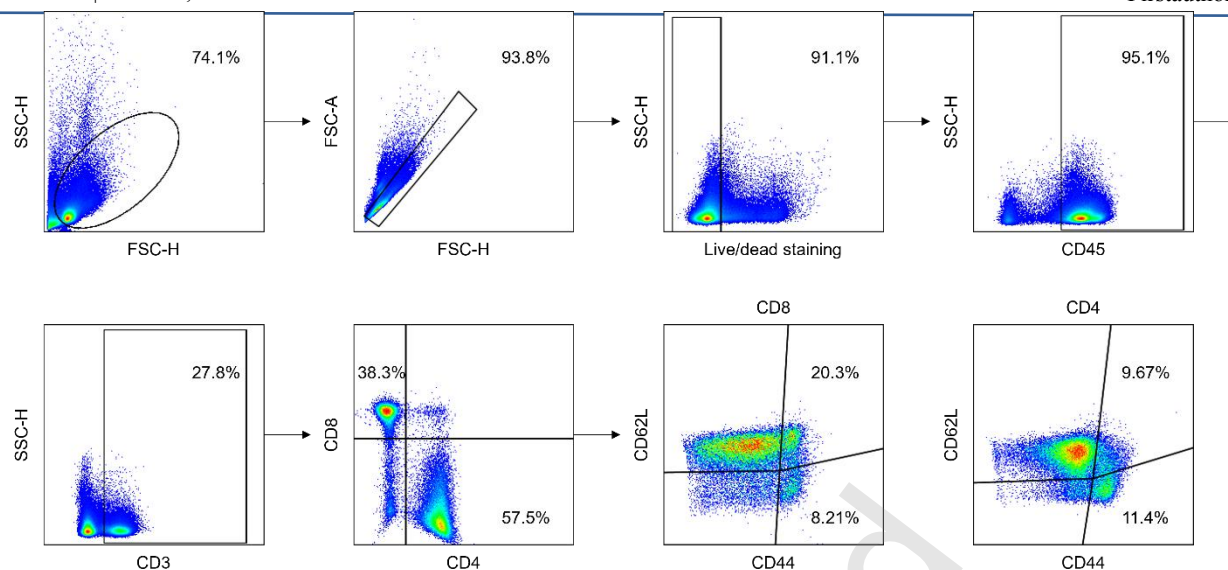


Fig. S28. FCM gating strategy for the analysis of CD44⁺ CD62L⁺ CD4⁺ and CD8⁺ T-cell populations in the prophylactic B16-OVA model.

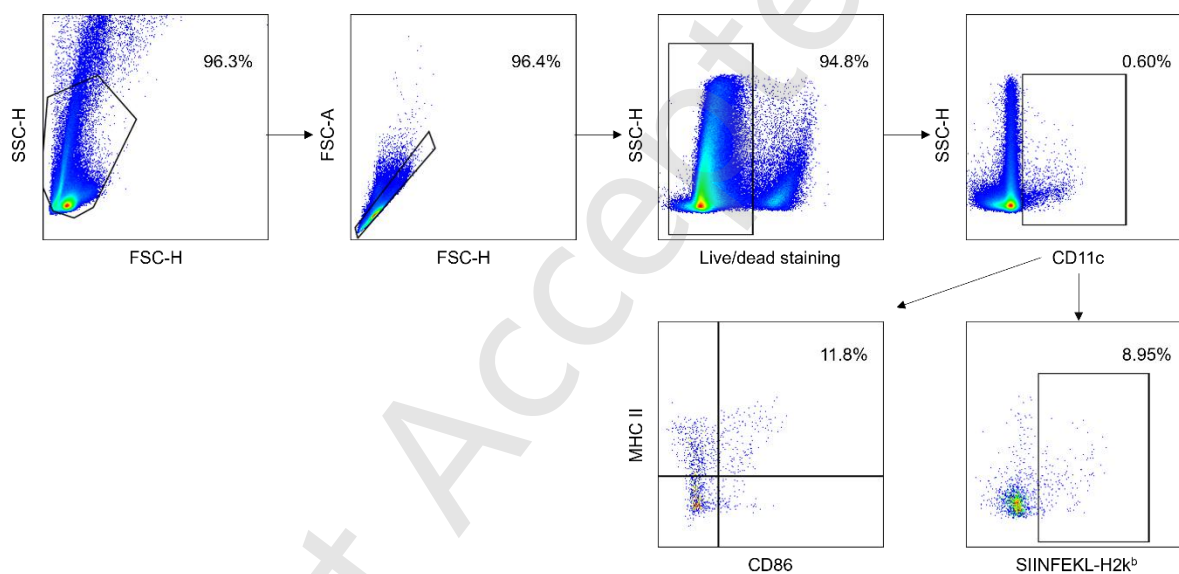


Fig. S29. FCM gating strategy for the analysis of CD86⁺ MHC II⁺ and SIINFEKL-H2K^b⁺ cells within the CD11c⁺ population in lymph-node cells in the prophylactic B16-OVA model.