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Enhancing the immunogenicity of lipid-nanoparticle mRNA vaccines by adjuvanting the ionizable lipid and the mRNA

In the format provided by the authors and unedited

Contents

Supplementary Methods | CDS of all constructs in this study.

CDS of SP from wild-type SARS-CoV-2:

atgtttgtttttcttgttttattgccactagtctctagtcagtgtgttaatcttacaaccagaactcaattacccccctgcatacactaattctttcac
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CDS of SP from wild-type SARS-CoV-2 fused to C3d (SP-C3d)

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CDS of RBD from wild-type SARS-CoV-2

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gggatccggttaa

CDS of RBD from wild-type SARS-CoV-2 fused to C3d (RBD-C3d):

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CDS of RBD from Delta Variant SARS-CoV-2:

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aagggatccggttaa

CDS of RBD from Delta Variant SARS-CoV-2 fused to C3d (RBD Delta-C3d)

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taa

CDS of C3d alone

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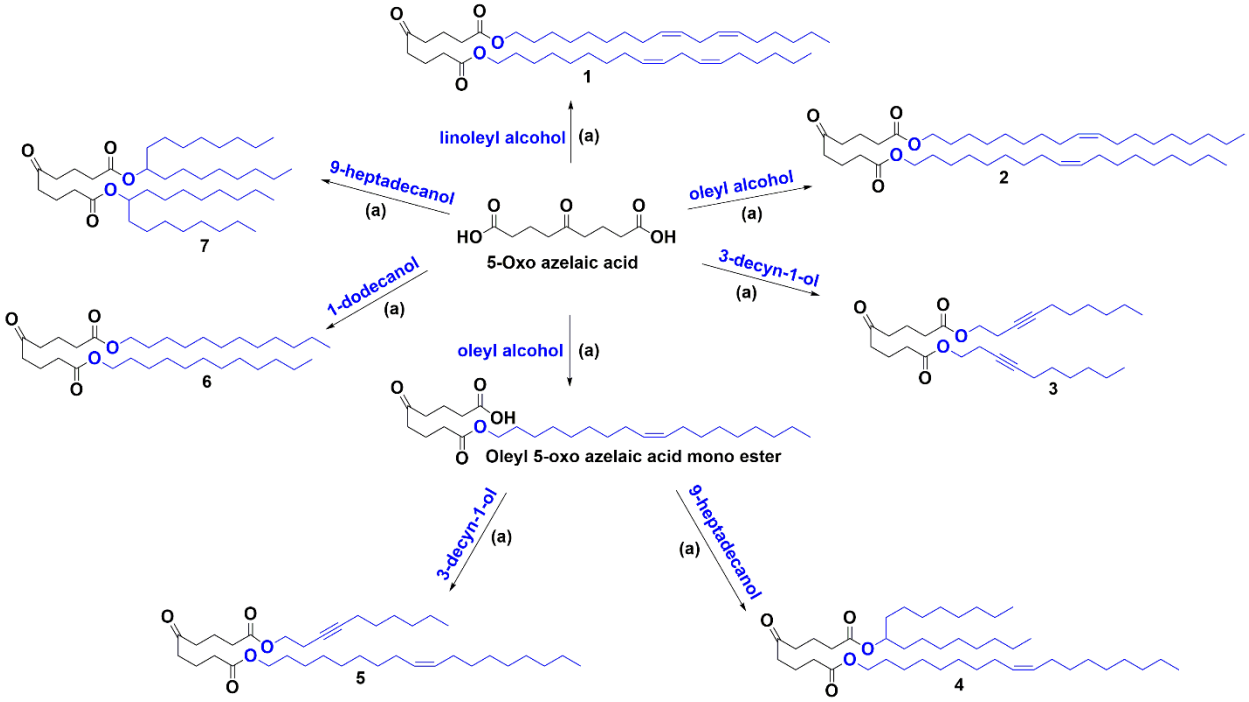
Supplementary Methods I Synthesis and characterization of combinatorial ionizable lipid library.

All solvents and reagents were obtained commercially and used unless noted otherwise. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at room temperature using a Bruker Ultrashield 400 MHz instrument. Spectra were processed with Chemical shifts reported as parts per million (ppm) relative to TMS (0.00) for ^{1}H. Silica gel chromatography was performed on ISCO CombiFlash Rf+ Lumen Instruments using ISCO RediSep Rf Gold Flash Cartridges (particle size: 20-40 microns). All final compounds were confirmed by mass spectrometry using a direct injection method on QTOF-HRMS (Agilent) coupled to an Agilent Infinity 1260 LC system.



Scheme 1: Synthesis of tails used for lipid synthesis. (a) EDC, DIPEA, DMAP, DCM

Method A: Synthesis of Oleyl 5-oxo azelaic acid monoester

Oxo azalaic acid (2.0g, 9.9 mmol), oleyl alcohol (1.3g, 4.9 mmol), N-(3-dimethylaminopropyl)- N′-ethylcarbodiimide hydrochloride (EDC HCl, 0.9g, 4.9 mmol), 4-(dimethylamino) pyridine (DMAP, 0.3g, 2.5 mmol) and N, N-diisopropylethylamine (DIPEA, 0.8 ml, 4.9 mmol) were dissolved in dichloromethane (100 ml). The reaction was stirred at room temperature under nitrogen for 18 h. The reaction solvent was evaporated under vacuum, and the residue purified by silica gel chromatography (0–10% methanol in dichloromethane) to give oleyl 5-oxo azelaic acid mono ester (1.6g, 1.6 mmol, 35%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 – 5.26 (m, 2H), 4.07 (t, J = 6.8 Hz, 2H), 2.51 (q, J = 7.5 Hz, 4H), 2.41 (t, J = 7.2 Hz, 2H), 2.34 (t, J = 7.2 Hz, 2H), 2.03 (q, J = 6.4 Hz, 3H), 1.92 (pd, J = 7.2, 4.6 Hz, 4H), 1.64 (q, J = 6.9 Hz, 2H), 1.30 (q, J = 7.8, 4.8 Hz, 23H), 0.90 (t, J = 6.7 Hz, 3H).

Method B: Synthesis of Tail **1** di((9Z,12Z)-octadeca-9,12-dien-1-yl) 5-oxononanedioate

Oxo azalaic acid (1g, 4.95 mmol), N-(3-dimethylaminopropyl)- N′-ethylcarbodiimide hydrochloride (EDC HCl, 2.8g, 12.4 mmol), linoleyl alcohol (4.0g, 14.8 mmol), 4-(dimethylamino) pyridine (DMAP, 0.3g, 2.5 mmol) and N, N-diisopropylethylamine (DIPEA, 2.6 ml, 14.8 mmol) were dissolved in dichloromethane (100 ml). The reaction was stirred at room temperature under

nitrogen for 18 h, then washed with a saturated aqueous sodium bicarbonate solution. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered, and the filtrate was evaporated under vacuum. The residue was purified by silica gel chromatography (0–50% ethyl acetate in hexanes) to give tail **1**, (2.0g, 2.9 mmol, 59%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38 (m, J = 11.0, 6.0 Hz, 8H), 4.07 (t, J = 6.8 Hz, 4H), 2.80 (t, J = 6.6 Hz, 4H), 2.49 (t, J = 7.2 Hz, 4H), 2.34 (t, J = 7.3 Hz, 4H), 2.07 (q, J = 7.0 Hz, 8H), 1.91 (p, J = 7.3 Hz, 4H), 1.63 (t, J = 7.0 Hz, 6H), 1.43 – 1.24 (m, 31H), 0.91 (t, J = 6.7 Hz, 6H).

Synthesis of Tail **2** di((Z)-octadec-9-en-1-yl) 5-oxononanedioate:
Made using method B as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44 – 5.26 (m, 4H), 4.07 (t, J = 6.8 Hz, 4H), 2.49 (t, J = 7.2 Hz, 4H), 2.34 (t, J = 7.2 Hz, 4H), 2.03 (q, J = 6.5 Hz, 7H), 1.91 (p, J = 7.3 Hz, 4H), 1.62 (q, J = 6.9 Hz, 6H), 1.44 – 1.20 (m, 43H), 0.90 (t, J = 6.7 Hz, 6H).

Synthesis of Tail **3** di(dec-3-yn-1-yl) 5-oxononanedioate:
Made using method B as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (t, J = 7.0 Hz, 4H), 2.48 (td, J = 7.1, 3.7 Hz, 8H), 2.34 (t, J = 7.2 Hz, 4H), 2.13 (tt, J = 7.1, 2.4 Hz, 4H), 1.89 (p, J = 7.2 Hz, 4H), 1.51 – 1.41 (m, 4H), 1.41 – 1.20 (m, 12H), 0.88 (t, J = 6.9 Hz, 6H).

Method C: synthesis of Tail **4** (Z)-1-(heptadecan-9-yl) 9-(octadec-9-en-1-yl) 5-oxononanedioate:
Oleyl 5-oxo azelaic acid mono ester (0.18 g, 0.40 mmol), N-(3-dimethylaminopropyl)- N′-ethylcarbodiimide hydrochloride (EDC HCl, 0.11g, 0.60 mmol), 9-heptadecanol (0.11g, 0.44 mmol), 4-(dimethylamino) pyridine (DMAP, 0.03g, 0.2 mmol) and N, N-diisopropylethylamine (DIPEA, 0.14 ml, 0.80 mmol) were dissolved in dichloromethane (5 ml). The reaction was stirred at room temperature under nitrogen for 18 h, then washed with a saturated aqueous sodium bicarbonate solution. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered, and the filtrate was evaporated under vacuum. The residue was purified by silica gel chromatography (0–50% ethyl acetate in hexanes) to give tail **4**, (0.17g, 0.25 mmol, 63%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 – 5.28 (m, 2H), 4.88 (p, J = 6.3 Hz, 1H), 4.07 (t, J = 6.8 Hz, 2H), 2.49 (t, J = 7.2 Hz, 4H), 2.33 (td, J = 7.2, 4.9 Hz, 4H), 2.03 (q, J = 6.4 Hz, 4H), 1.92 (q, J = 7.3 Hz, 4H), 1.62 (m, J = 6.0 Hz, 3H), 1.52 (q, J = 6.1 Hz, 4H), 1.43 – 1.17 (m, 46H), 0.90 (t, J = 6.7 Hz, 9H).

Synthesis of Tail **5** (Z)-1-(dec-3-yn-1-yl) 9-(octadec-9-en-1-yl) 5-oxononanedioate:
Made using method C as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43 – 5.30 (m, 2H), 4.15 (t, J = 7.0 Hz, 2H), 4.07 (t, J = 6.8 Hz, 2H), 2.50 (m, J = 7.1, 2.8 Hz, 6H), 2.35 (m, J = 10.0, 7.2 Hz, 4H), 2.15 (m, J = 7.2, 2.4 Hz, 2H), 2.03 (q, J = 6.5 Hz, 3H), 1.91 (m, J = 7.3, 2.7 Hz, 4H), 1.68 – 1.56 (m, 4H), 1.54 – 1.43 (m, 2H), 1.43 – 1.22 (m, 27H), 0.91 (m, J = 6.9, 3.2 Hz, 6H).

Synthesis of Tail **6 dodecyl** 5-oxononanedioate:
Made using method B as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07 (t, J = 6.8 Hz, 4H), 2.49 (t, J = 7.2 Hz, 4H), 2.34 (t, J = 7.2 Hz, 4H), 1.91 (p, J = 7.2 Hz, 4H), 1.69 – 1.57 (m, 4H), 1.28 (s, 36H), 0.90 (t, J = 6.7 Hz, 6H).

Synthesis of Tail **7** di(heptadecan-9-yl) 5-oxononanedioate:
Made using method B as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.87 (p, J = 6.3 Hz, 2H), 2.48 (t, J = 7.3 Hz, 4H), 2.32 (t, J = 7.3 Hz, 4H), 1.90 (p, J = 7.3 Hz, 4H), 1.52 (q, J = 6.1 Hz, 8H), 1.27 (m, 48H), 0.89 (t, J = 6.7 Hz, 12H).

For high throughput synthesis:
10 μL of 350 μM stock solutions of amines, linkers, and tails in a 2:1 mixture of dichloromethane and ethanol were added to each well of a 96-well plate with glass inserts. The plates were covered and placed on a shaker to stir overnight. Lipids were formulated into LNP in the same reaction plates.

Synthesis of Lipid **331** di((9Z,12Z)-octadeca-9,12-dien-1-yl) 4,4'-(2-(tert-butoxycarbonyl)-1-(2-(2-ethylpiperidin-1-yl)ethyl)-2,5-dihydro-1H-imidazole-5,5-diyl)dibutyrate:
Di((9Z,12Z)-octadeca-9,12-dien-1-yl) 5-oxononanedioate, tail **1** (100 mg, 0.14mmol), 2-(2-ethylpiperidin-1-yl)ethan-1-amine (22 mg, 0.14 mmol), and tert-butyl isocyanoacetate (20 mg, 0.14 mmol) were dissolved in a mixture of anhydrous solvents consisting of dichloromethane (DCM) 120μL and ethanol 80μL a in capped glass vial. The reaction was stirred at room temperature overnight. The mixture was purified by silica gel chromatography (10% methanol in dichloromethane with 0.1% NH_4OH) to give lipid **331** as a brown oil (20mg, 0.02 mmol, 14%). QTOF MS (ESI): m/z calcd for $C_{61}H_{108}N_3O_6^+$ (M+H), 978.82326; found, 978.8233. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (dd, J = 14.4, 1.9 Hz, 1H), 5.49 – 5.26 (m, 8H), 4.43 (s, 1H), 4.05 (dt, J = 12.0, 6.8 Hz, 4H), 3.16 (ddd, J = 14.1, 9.9, 6.6 Hz, 1H), 2.99 (ddt, J = 13.4, 8.6, 4.8 Hz, 1H), 2.94 – 2.72 (m, 6H), 2.56 – 2.41 (m, 2H), 2.30 (ddt, J = 23.2, 13.1, 3.8 Hz, 5H), 2.19 – 1.98 (m, 9H), 1.83 – 1.43 (m, 28H), 1.43 – 1.21 (m, 34H), 1.01 – 0.82 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.17, 173.11, 170.31, 156.55, 130.21, 130.06, 128.04, 127.91, 81.45, 74.25, 74.17, 70.04, 69.99, 64.62, 64.56, 61.99, 61.84, 53.85, 53.73, 51.72, 51.51, 39.65, 39.33, 37.97, 37.87, 34.62, 34.56, 34.23, 34.17, 31.53, 29.64, 29.52, 29.44, 29.39, 29.35, 29.29, 29.25, 29.00, 28.65, 28.63, 28.03, 27.20, 25.92, 25.91, 25.63, 25.31, 25.26, 23.00, 22.70, 22.57, 19.73, 19.70, 19.34, 14.07, 10.34, 10.13.

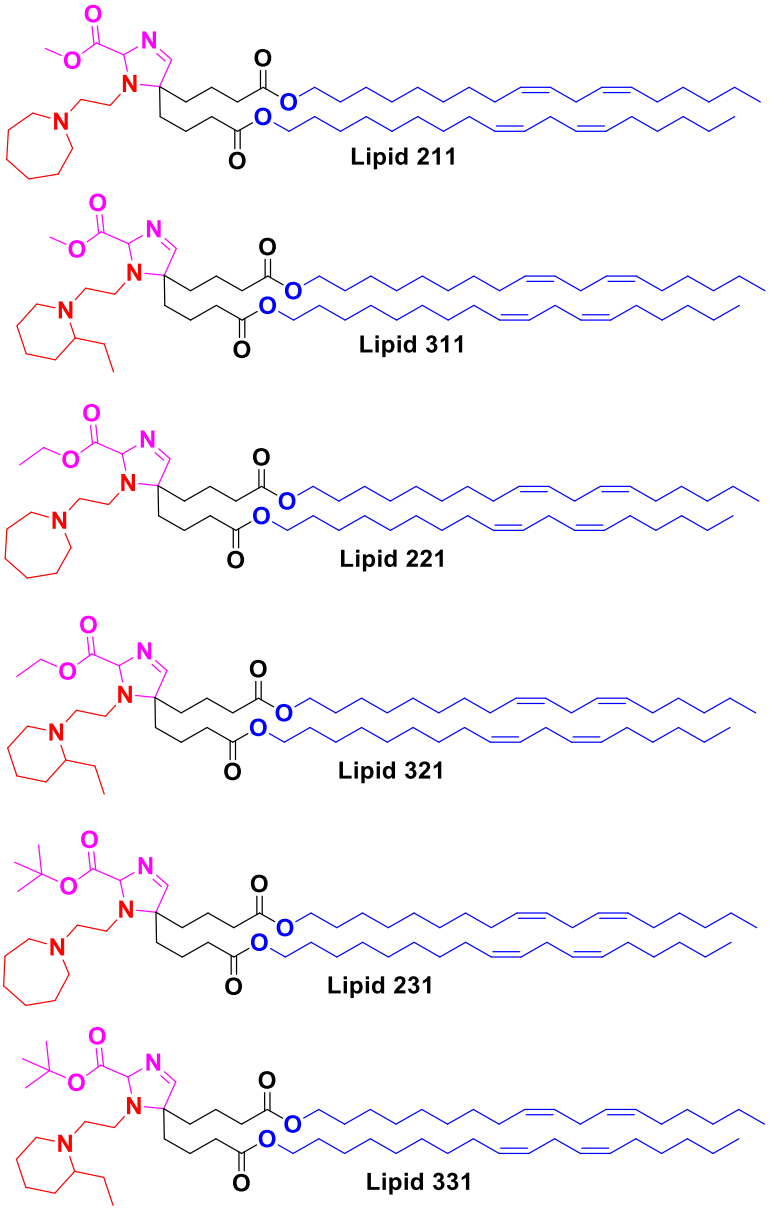
Synthesis of Lipid **221** di((9Z,12Z)-octadeca-9,12-dien-1-yl) 4,4'-(1-(2-(azepan-1-yl)ethyl)-2-(ethoxycarbonyl)-2,5-dihydro-1H-imidazole-5,5-diyl)dibutyrate:
Lipid **221** was made following the same procedure for Lipid **331** above using di((9Z,12Z)-octadeca-9,12-dien-1-yl) 5-oxononanedioate, tail **1** (100 mg, 0.14mmol), 2-(azepan-1-yl)ethan-1-amine (20 mg, 0.14 mmol), and ethyl isocyanoacetate (16 mg, 0.14 mmol) to give lipid **221** as a brown oil (12mg, 0.013 mmol, 9%). QTOF MS (ESI): m/z calcd for $C_{58}H_{102}N_3O_6^+$ (M+H), 936.77631; found, 936.7761. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 1H), 5.47 – 5.27 (m, 8H), 4.55 (s, 1H), 4.27 (qd, J = 7.2, 2.6 Hz, 2H), 4.06 (dt, J = 13.9, 6.9 Hz, 4H), 3.13 (dt, J = 13.5, 6.6 Hz, 1H), 2.99 (dt, J = 14.0, 6.1 Hz, 1H), 2.79 (t, J = 6.5 Hz, 4H), 2.68 (dq, J = 11.6, 7.8, 7.0 Hz, 7H), 2.34 (dq, J = 10.8, 9.2, 7.9 Hz, 2H), 2.07 (q, J = 6.8 Hz, 8H), 1.74 – 1.52 (m, 21H), 1.42 – 1.21 (m, 36H), 0.91 (t, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.21, 173.11, 171.56, 156.70, 130.23, 130.07, 128.05, 127.92, 73.64, 69.94, 64.65, 64.57, 60.99, 58.34, 55.71, 55.16, 39.79, 37.76, 34.46, 34.25, 34.08, 31.54, 29.66, 29.45, 29.36, 29.26, 28.66, 28.64, 28.31, 27.23, 27.21, 26.93, 26.86, 25.94, 25.92, 25.64, 22.58, 19.50, 19.28, 14.24, 14.08.

Synthesis of Lipid **321** di((9Z,12Z)-octadeca-9,12-dien-1-yl) 4,4'-(2-(ethoxycarbonyl)-1-(2-(2-ethylpiperidin-1-yl)ethyl)-2,5-dihydro-1H-imidazole-5,5-diyl)dibutyrate:
Lipid 3**21** was made following the same procedure for Lipid **331** above using di((9Z,12Z)-octadeca-9,12-dien-1-yl) 5-oxononanedioate, tail **1** (100 mg, 0.14mmol), 2-(2-ethylpiperidin-1-yl)ethan-1-amine (22 mg, 0.14 mmol), and ethyl isocyanoacetate (16 mg, 0.14 mmol) to give lipid **321** as a brown oil (15mg, 0.02 mmol, 14%). QTOF MS (ESI): m/z calcd for $C_{59}H_{104}N_3O_6^+$ (M+H), 950.79196; found, 950.7922. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (s, 1H), 5.37 (pd, J = 10.4, 9.0, 5.2 Hz, 8H), 4.54 (s, 1H), 4.27 (qq, J = 6.5, 3.6 Hz, 2H), 4.05 (dt, J = 13.9, 6.8 Hz, 4H), 3.15 (ddd, J = 19.1, 13.8, 7.0 Hz, 1H), 2.99 (ddd, J = 18.2, 9.3, 5.3 Hz, 1H), 2.92 – 2.72 (m, 6H), 2.52 – 2.38

(m, 3H), 2.37 – 2.29 (m, 3H), 2.24 (tt, J = 10.1, 5.2 Hz, 3H), 2.07 (q, J = 6.9 Hz, 8H), 1.76 – 1.52 (m, 18H), 1.41 – 1.23 (m, 36H), 0.89 (dt, J = 11.5, 7.1 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.16, 173.05, 171.46, 156.75, 130.23, 130.07, 128.04, 127.91, 73.82, 73.73, 70.08, 70.02, 64.64, 64.57, 61.98, 61.85, 61.00, 53.90, 53.75, 51.70, 51.49, 39.64, 39.36, 37.92, 37.81, 34.49, 34.42, 34.29, 34.12, 31.53, 29.77, 29.65, 29.53, 29.45, 29.35, 29.32, 29.26, 29.08, 28.65, 28.64, 27.22, 27.21, 25.97, 25.93, 25.91, 25.63, 25.37, 25.34, 23.02, 22.74, 22.58, 19.60, 19.32, 14.23, 14.12, 14.08, 10.35, 10.16.

Synthesis of Lipid **231** di((9Z,12Z)-octadeca-9,12-dien-1-yl) 4,4'-(1-(2-(azepan-1-yl)ethyl)-2-(tert-butoxycarbonyl)-2,5-dihydro-1H-imidazole-5,5-diyl)dibutyrate:

Lipid **231** was made following the same procedure for Lipid **331** above using di((9Z,12Z)-octadeca-9,12-dien-1-yl) 5-oxononanedioate, tail **1** (100 mg, 0.14mmol), 2-(azepan-1-yl)ethan-1-amine (20 mg, 0.14 mmol), and tert-butyl isocyanoacetate (20 mg, 0.14 mmol) to give lipid **231** as a brown oil (10mg, 0.01 mmol, 7%). QTOF MS (ESI): m/z calcd for $C_{60}H_{106}N_3O_6^+$ (M+H), 964.80761; found, 964.8073. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 1H), 5.48 – 5.28 (m, 8H), 4.44 (s, 1H), 4.05 (dt, J = 12.2, 6.8 Hz, 4H), 3.14 (dt, J = 13.6, 6.7 Hz, 1H), 2.97 (dt, J = 13.9, 6.1 Hz, 1H), 2.79 (t, J = 6.4 Hz, 4H), 2.68 (dq, J = 10.2, 5.4, 4.5 Hz, 5H), 2.42 – 2.23 (m, 4H), 2.23 – 2.12 (m, 1H), 2.07 (q, J = 6.9 Hz, 8H), 1.78 – 1.55 (m, 18H), 1.52 (s, 10H), 1.45 – 1.24 (m, 33H), 0.91 (t, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.23, 173.18, 170.49, 156.47, 130.23, 130.07, 128.04, 127.91, 81.36, 74.26, 69.83, 64.62, 64.56, 58.37, 55.71, 39.83, 37.83, 34.62, 34.23, 34.17, 31.53, 29.65, 29.45, 29.35, 29.32, 29.25, 28.66, 28.64, 28.27, 28.05, 27.22, 27.21, 26.94, 25.93, 25.92, 25.63, 22.58, 19.66, 19.33, 14.08.

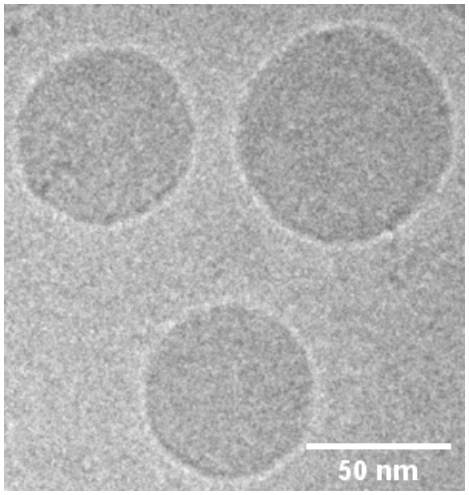


Supplementary Fig. 1 | Structures of top-performing lipids identified from our screen.

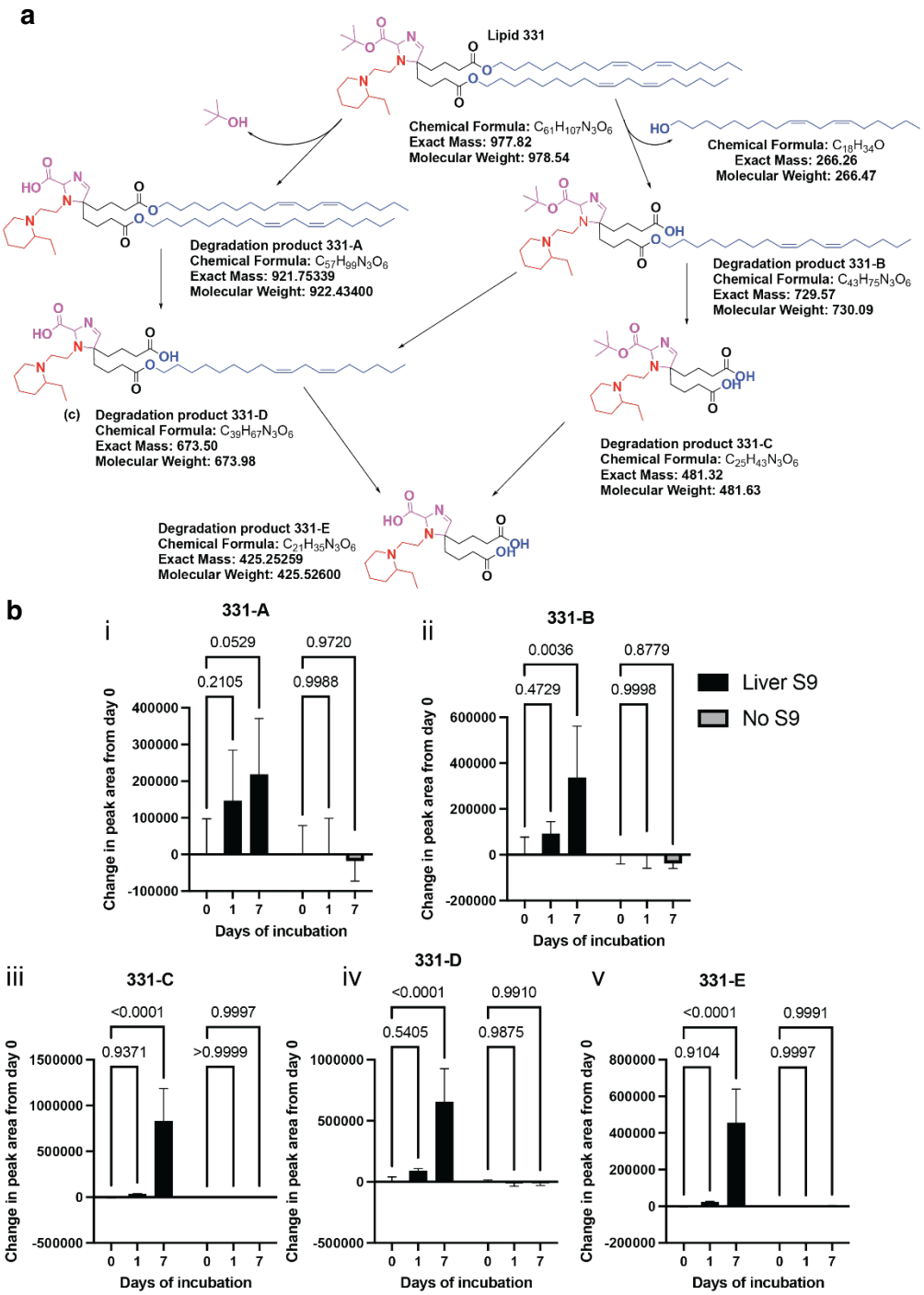
a

LNP	Size (nm)	PDI	Zeta Potential (mv)	Encapsulation efficiency (%)	LNP pKa
Lipid 211	61.2 ± 1.0	0.2	1.4 ± 0.8	84.9 ± 7.4	-
Lipid 311	56.7 ± 1.5	0.2	2.5 ± 0.7	85.0 ± 4.7	-
Lipid 221	69.7 ± 1.6	0.2	-1.4 ± 3.3	91.1 ± 8.3	-
Lipid 321	59.8 ± 4.0	0.2	-2.7 ± 1.9	83.5 ± 8.2	-
Lipid 231	53.1 ± 1.4	0.2	-6.1 ± 0.8	82.4 ± 6.7	-
Lipid 331	65.4 ± 0.8	0.2	-6.2 ± 1.7	75.0 ± 8.9	7.53 ± 0.17
MC3	75.3 ± 0.4	0.1	-4.9 ± 1.7	70.0 ± 7.8	6.68 ± 0.06
ALC-0315	101.6 ± 0.5	0.2	-1.5 ± 0.8	80.3 ± 8.6	6.77 ± 0.06
A18-Iso5-2DC18	101.7 ± 0.7	0.2	0.3 ± 3.0	72.3 ± 9.8	7.75 ± 0.23

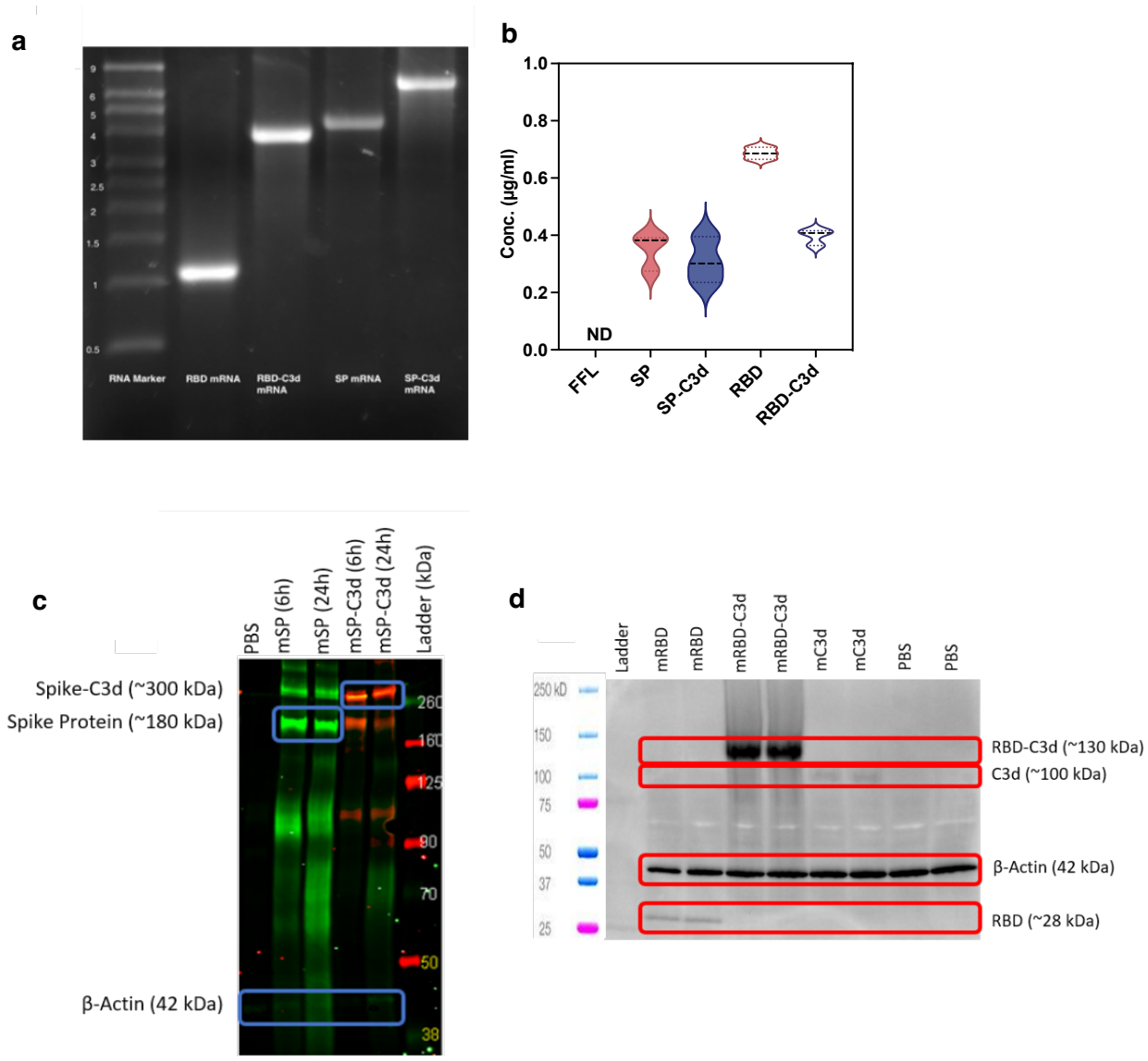
b



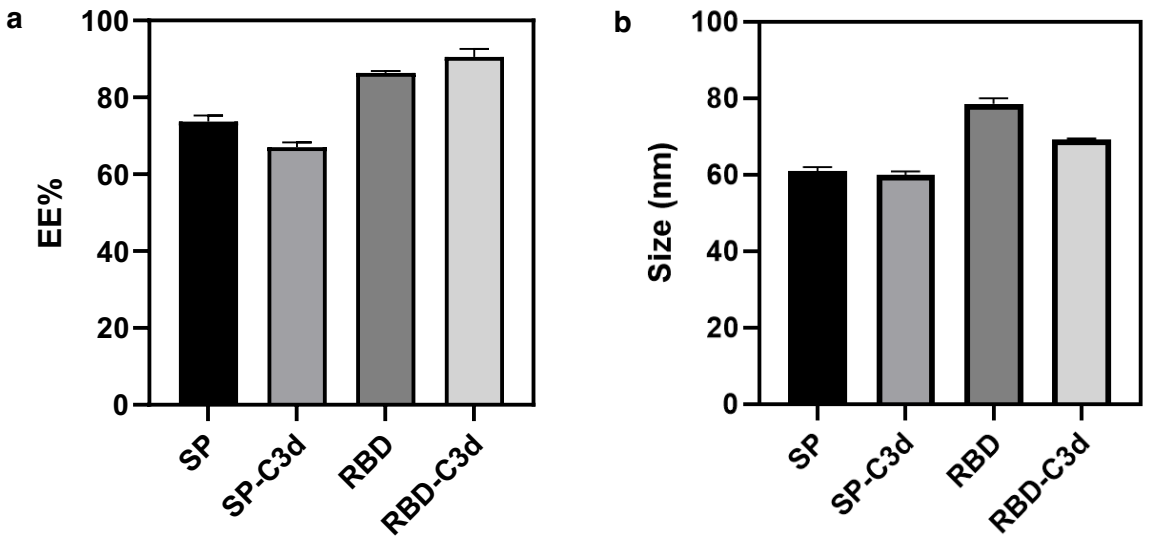
Supplementary Fig. 2 | Physiochemical characterization of top-performing immunostimulatory LNPs. a, Physiochemical properties of top performing LNPs and control LNPs. n = 3 repeated measurements, data are presented as mean ± SD. **b,** Cryogenic transmission electron microscopy (cryoTEM) imaging of LNPs containing lipid 331. Scale bar indicates 50 nm.



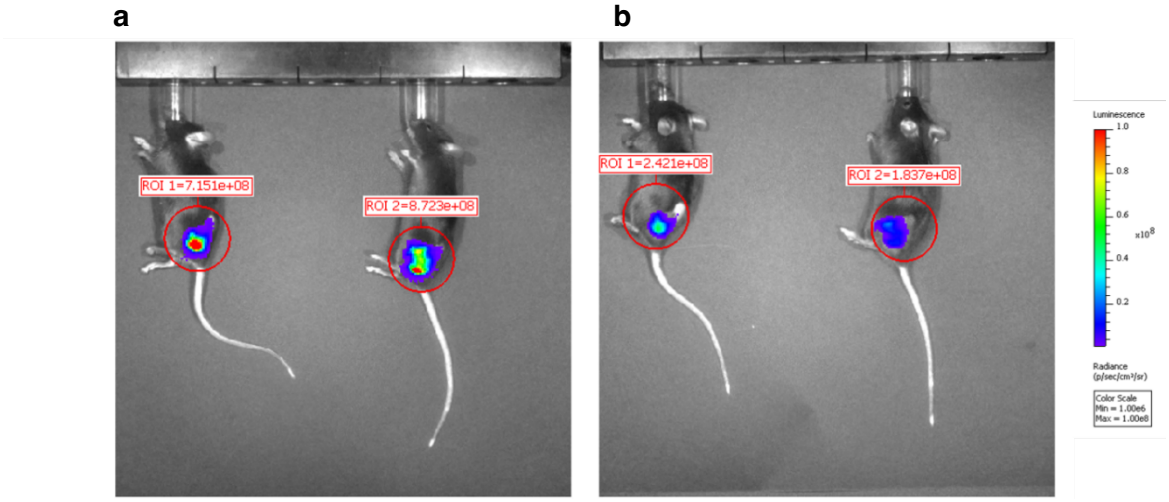
Supplementary Fig. 3 | Proposed esterase-mediated degradation pathways for lipid 331. a) Degradation can occur via cleavage of *tert*-butyl linker and/or linoleic tail. Possible degradation products labeled A through E. b) Change in area of LCMS peak corresponding to each degradation product after 0, 1, or 7 days of incubation with 2mg/mL human liver S9 fraction or PBS control. Data are presented as mean±SD (n = 3), and statistical significance was analyzed by using a one-way ANOVA with post-hoc Tukey test.



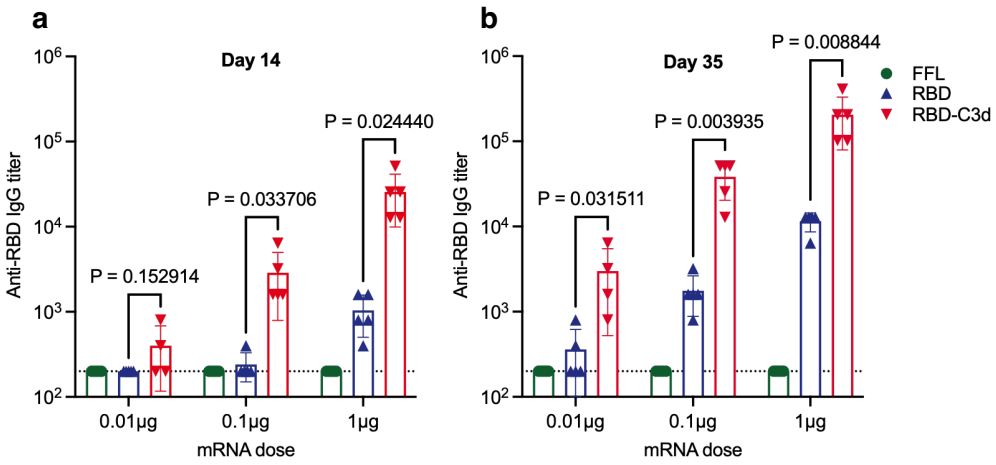
Supplementary Fig. 4 | Characterization of mRNA and encoded proteins. a, Analysis of mRNA by gel electrophoresis. 1.5μg of mRNA was loaded into each lane. **b,** SP, SP-C3d, RBD, and RBD-C3d expression in transfected HEK293T cells determined by ELISA following transfection with 1μg of mRNA. **c,** Fluorescent western blot of denatured lysate samples from HEK293T cells transfected with mSP or mSP-C3d lysed at either 6h or 24h following transfection. Detection was performed by first incubating blots with rabbit anti-actin, rabbit anti-S2, and goat anti-C3d followed by incubation with secondary antibodies against rabbit (green) and goat (red) IgGs. Therefore, it is expected that actin and spike protein alone will appear green on the fluorescent blot while the spike-C3d fusion protein will appear orange (green + red simultaneously). **d,** Chemiluminescent western blot of denatured lysate samples from HEK293T cells transfected with mRBD, mRBD-C3d, or mC3d. All transfected groups run in duplicate with each lane representing an individual well of HEK293T cells transfected with mRNA. Detection was performed with antibodies against actin, RBD, and C3d.



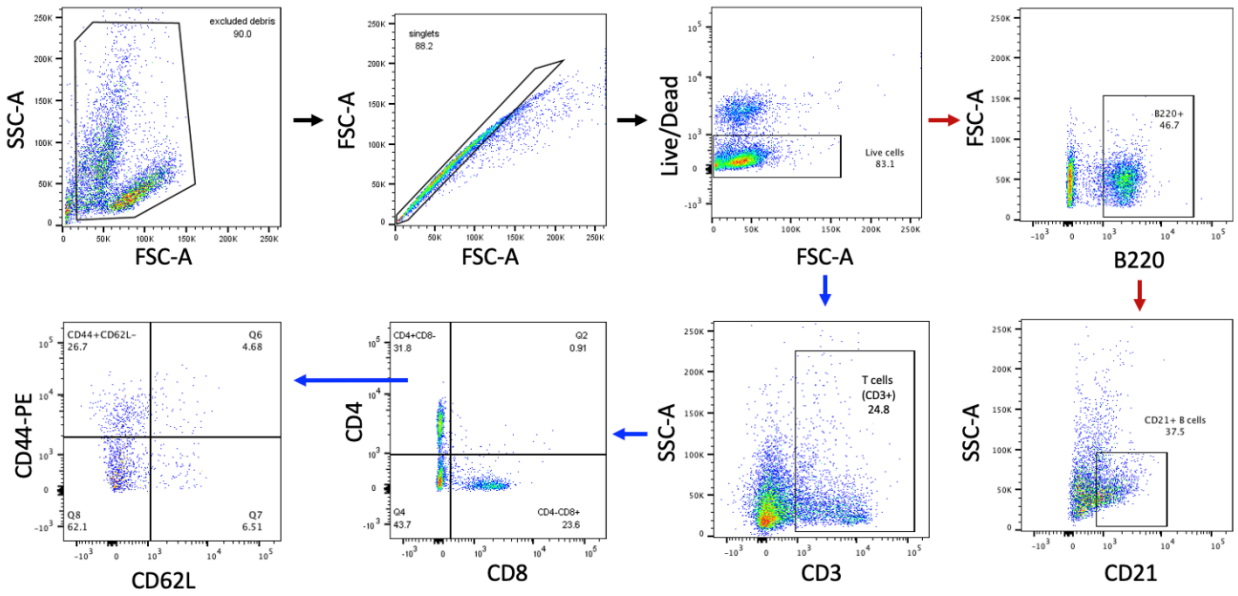
Supplementary Fig. 5 | Characterization of LNPs formulated with different mRNA encoding SP, SP-C3d, RBD, or RBD-C3d. a, Encapsulation efficiency (EE%). **b,** particle size measured by DLS.



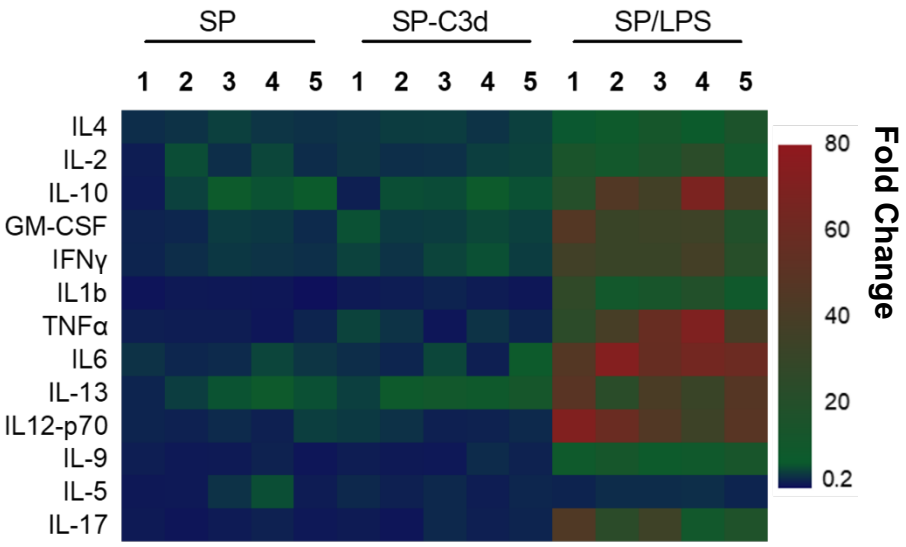
Supplementary Fig. 6 | IVIS imaging of mice intramuscularly dosed with cKKE-12 LNPs loaded with mFFL. cKK-E12 LNPs were injected intramuscularly into mice (0.25mg mRNA/kg mouse). The FFL expression was visualized at 6 h (a) and 24 h (b) after injection by IVIS imaging.



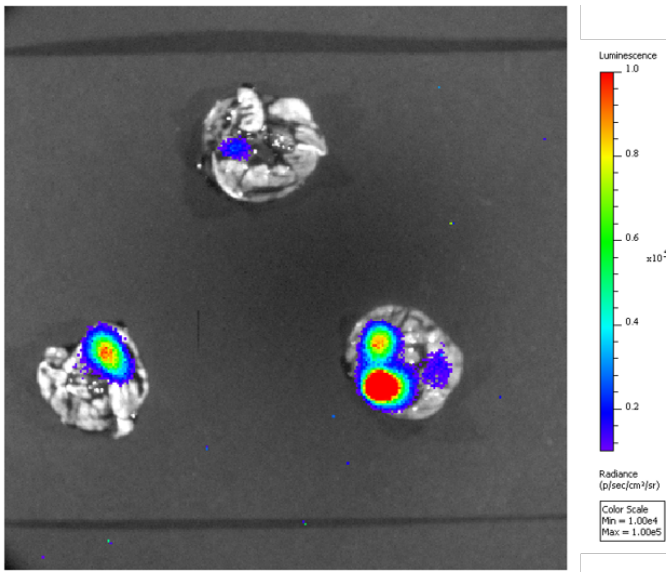
Supplementary Fig. 7 l Immunogenicity study of SARS-CoV-2 RBD mRNA with or without C3d fusion. C57BL/6J mice were immunized at weeks 0 and 3 with doses of LNP-formulated mRNA ranging from 0.01μg-1μg (n=5/group). Sera were collected on the 14th and 35th days post-prime. Titres of anti-RBD IgG were detected on days (**a**) 14 and (**b**) 35. Statistical significance was analyzed by a two-tailed Student's t-test.



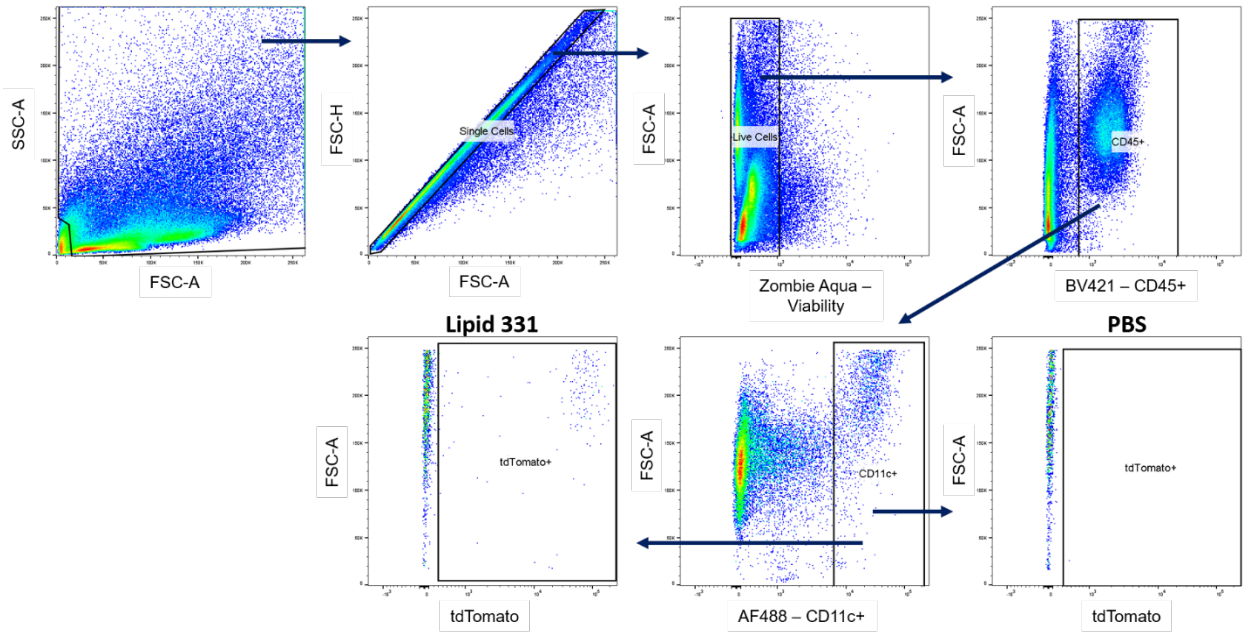
Supplementary Fig. 8. Gating strategy for flow cytometry analysis of T and B cells in C57/BL6 mice. Representative flow cytometry gating strategy to identify SARS-CoV-2 SP-specific $CD4^+$ T effector memory cells (Live/$CD3^+$/$CD4^+$$CD8^-$/$CD44^+$$CD62L^-$) and $CD21^+$ B cells (Live/$B220^+$/$CD21^+$) in splenocytes from mice vaccinated with cKK-E12 LNP/mSP-C3d (1µg mRNA per mouse) upon re-stimulation with SARS-CoV-2 peptide pools.



Supplementary Fig. 9 | Induction of cytokines and chemokines. Fold change in concentration of systemic cytokines and chemokines in mouse sera at 6h post-vaccination of SARS-CoV-2 vaccines (1μg mRNA per mouse) compared to that in untreated mouse sera (n=5, each column represents an individual mouse).

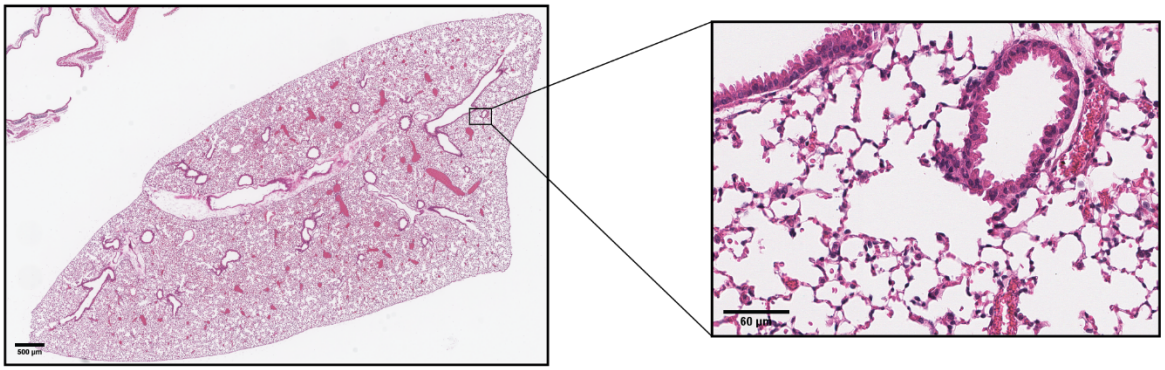


Supplementary Fig. 10 l IVIS imaging of mice intranasally dosed with lipid 331 LNPs loaded with mFFL. LNPs formulated with lipid 331 and mFFL were intranasally administered to mice (1μg/mouse). FFL expression in the lungs was visualized 6h after administration. Mouse lungs shown here are from the same mice shown in Figure 4a.

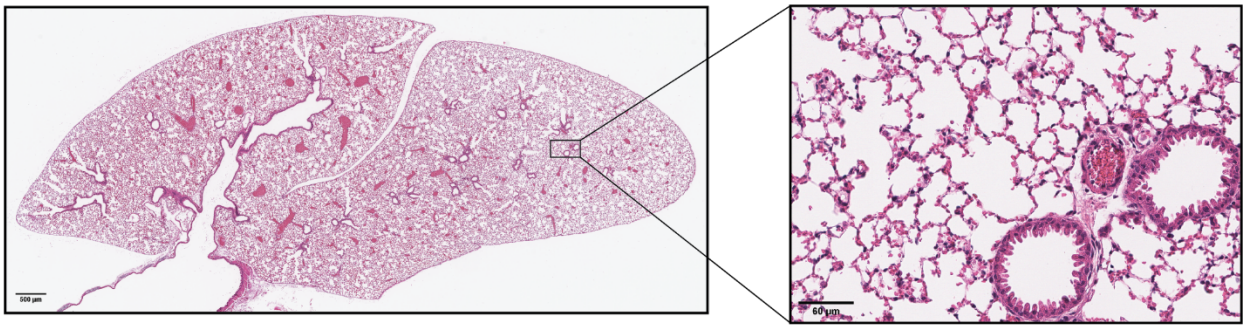


Supplementary Fig. 11 l Gating Strategy for flow cytometry analysis of lung-resident APCs in Ai14 mice. Representative flow cytometry gating strategy to identify tdTomato$^+$, CD11c$^+$ APCs following intranasal administration of 1μg mCre encapsulated in lipid 331 LNPs to Ai14 mice.

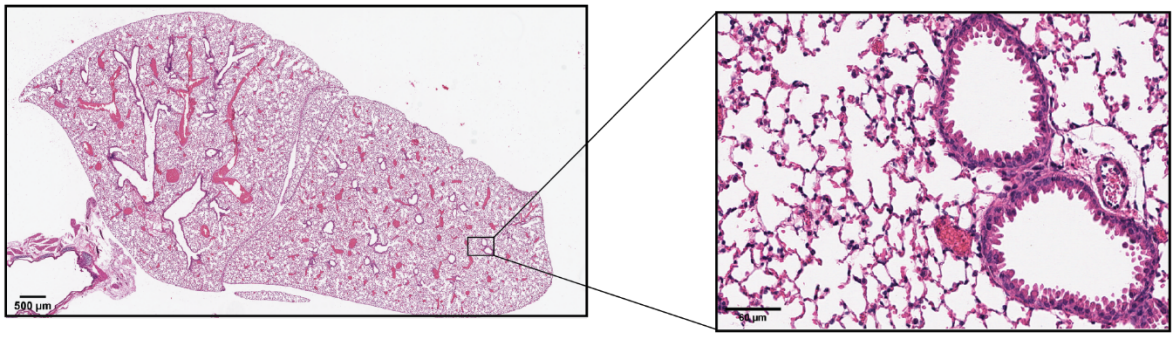
PBS



Lipid 331 - 1µg



Lipid 331 - 5µg



Supplementary Fig. 12 | Histology of mouse lung tissues. H&E staining of lungs harvested from mice 24h following intranasal administration of PBS or lipid 331 LNPs (1μg or 5μg dose of mFFL). Normal lung morphology was maintained in all groups as determined by a board-certified pathologist.

Competing Interests

For a list of entities with which R.L. is, or has been recently involved, compensated or uncompensated, please refer to the below list (accurate as of July 2023):

611 Therapeutics
Abpro International
Acorda (Formerly Civitas Therapeutics)
Alfred University
Aleph Farms
Alivio Therapeutics
Alkermes
Allevi
Allurion
Alnylam Pharmaceuticals, Inc
Amberstone Bioscience
Amgen
aMoon
Apotex
Arcadia Biosciences, Inc
Arsenal Medical
Artificial Cell Technology, Inc
Avalon-Globocare
Bai Biosciences
BASF Corporation
Bayer
Balzan Foundation
Bexson Biomedical
Bilayer Therapeutics
Biogen
BioInnovation Institute (Novo Nordisk Founden)
BioTE Medical
Blackrock
Blackstone (Formerly Clarus)
Boston Children's Hospital
CBC Group Investment Mgmt Group
Celanese
Celero
Cellink/BICO
Dispendix
Eagle Pharmaceuticals
Earli
Edigene Biotechnology, Inc.
Editas Medicine, Inc.
ELC (Estee Lauder Companies)
Eli Lilly
Eisai Inc.
Entrega
EpiBone
Establishment Labs, SA.
Everlywell
Evox Therapeutics, Ltd.
Fate
Flagship Pioneering
Frequency Therapeutics, Inc.
GeneLeap Biotech
Genemedicine Co Lmtd
GenScript USA Inc
Geneo Medicine
GENUV
Glaxosmithkline Llc
Glycobia
Glympse Bio
Goldman Sachs
Greenlight Biosciences
HCR (HealthCare Royalty Partners)
HKF DNA Technologies
Hopewell Therapeutics
Horizon Discovery Group Plc
Humacyte, Inc.
IBEX Pharmaceuticals, Inc.
Landsdowne Labs
LikeMinds
Lonza
Luminopia, Inc.
Luye (Shandong luye)
Lyndra Therapeutics
Lyra Therapeutics (Formerly "480 Biomedical")
Maurice Marie Janot Award 2020
McGovern Institute
Medikinetics Co., Ltd.
Merck
MGH Ragon Institute
Micelle
Moderna Therapeutics
Momenta
Muse Biotechnologies Inc.
Mylan
N2Tech
Nanobiosym
Nanobiotix
Neochromosone
Neoteny 4 LLP
NextRNA
Newbridge Ventures LLC
Noveome Biotherapeutics, Inc.
Novo Nordisk
Ohio State University
Olivo (acquired by Shiseido)
Ovid Therapeutics
Particles for Humanity
Pfizer, Inc.
Pioneer Hi-Bred International, Inc.
Placon Therapeutics
Polaris Partners
Secant Medical, Inc.
Seer, Inc.
Selecta Biosciences
Senses LLC
Setsuro Tech Inc.
Seventh Sense Biosystems, Inc.
Shenzhen Rice Life Technology, Ltd
Shire Ag
Sigilon
Sigma Aldrich Co. Llc
Sio2
Ske S.R.L.
Soil Culture Solutions Llc (Dba Soilcea)
Souffle Therapeutics
Solvandria Foundation
SQZ Biotechnologies
StemBioSys, Inc.
SuonoBio
T2 Biosystems
Taconic Biosciences, Inc. (formerly Taconic Farms)
Taiwania Capital (Bio-Asia Taiwan Symposium)
TARA
Tarveda Therapeutics
Teal Bio
Terasaki Institute
Tesio Pharmaceuticals
Third Rock Ventures
Tiba Biotech LLC
TissiuM (formerly"Gecko")
Transgenic Inc.
Translate Bio (Formerly Rana Therapeutics, Inc.)

Cellomics Technology, Llc
Cellular Biomedical
CE&N/ ACS
Charles River Laboratories, Inc.
Clontech Laboratories
Combined Therapeutics ("CTx")
Conference Forum
Cornell University
Crispr Therapeutics Ag
Crown Bioscience Inc.
Daré Biosciences (Formerly Microchips Biotech, Juniper Pharmaceuticals, and Columbia Laboratories)
Daros, Inc.
DeepBiome
Dewpoint Therapeutics
Immunai
ImmuneXcite Inc.
Institute of Immunology Co. Ltd
Integrated DNA Technologies, Inc.
InVivo Therapeutics
IxBio
J.R. Simplot Company
Jnana Therapeutics
Kala Pharmaceuticals
Kallyope, Inc.
Kendall Capital
Kensa
Kodikaz Therapeutics
KAST (Korean Academy of Science and Technology)
Ksq Therapeutics, Inc.
Kunlun Capital
Pontifical Academy of Sciences
Portal Instruments
Preceres, Llc (Acquired by Monsanto)
PrognomIQ Inc.
Pulmatrix
PureTech
Quris
ReLive
Rensselaer Polytechnic Institute/ Department of Chemical and Biological Engineering
Reprocell Usa, Inc. (Formerly Stemgent)
Replay Bio
Rubius Therapeutics
Satellite Bio
SBEF (Seoul Bio Economy Forum)
Trilink Biotechnologies, Inc.
Unilever (Living Proof)
University of Bergen, Norway (Falch Lecture Honorarium)
VasoRX
Verseau Therapeutics, Inc.
Virex Health
Vitakey
Vivtex Corporation
Westlake University
Whitehead Institute
Wiki Foods
Xenter
YourBio (Formerly 7th Sense Biosystems)
Yz Biosciences (Guangzhou) Inc.
Zenomics
ZWI Therapeutics