Supplementary Information for

Combinatorial design of nanoparticles for pulmonary mRNA delivery and genome editing

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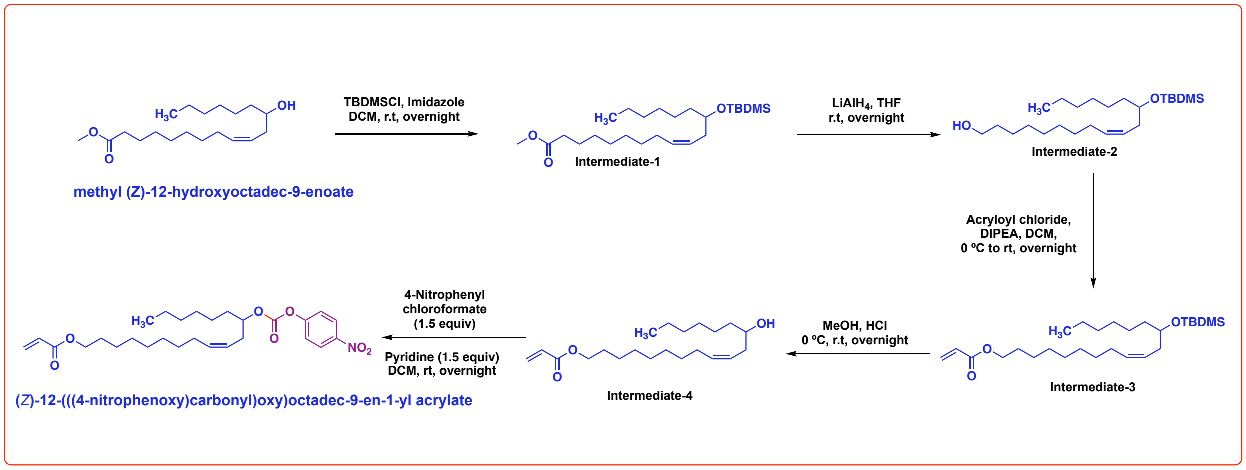
Supplementary Notes

Unless otherwise noted, all manipulations were carried out under an Argon atmosphere using standard Schlenk-line techniques. All glassware was oven-dried at 150 ºC overnight prior to use. Anhydrous THF, DCM, DMF, toluene, and ether were purchased from Sigma Aldrich as a sure seal bottle and used as received under Argon atmosphere. All the solvents used in synthesizing tails are anhydrous solvents. Amines are purchased from Sigma-Aldrich, TCI America, Alfa Aesar, and Ambeed. All other starting materials are obtained from commercial sources and were used as received. High throughput reactions were carried out in 96-well deep-well plates with glass inserts. 1H NMR spectra were obtained on a 500 MHz spectrometer, and chemical shifts were recorded relative to the residual protiated solvent. 13C NMR spectra were obtained on a 125 MHz spectrometer, and chemical shifts were recorded to the solvent resonance. Both 1H and 13C NMR chemical shifts were reported in parts per million downfield from tetramethylsilane (δ = 0). High-resolution mass spectra were obtained at a BioTOf HRMS at the DCIF facility.

Scheme for the synthesis of (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate:



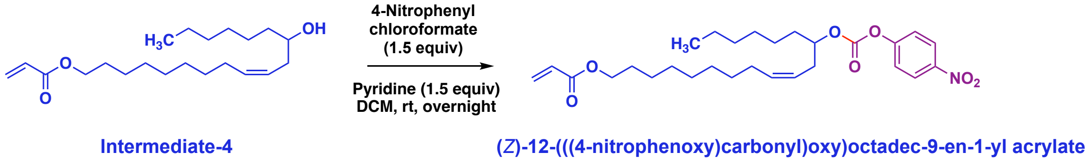
Intermediate-1: To an oven dried 100 mL round-bottom flask equipped with a magnetic stir bar taken methyl (*Z*)-12-hydroxyoctadec-9-enoate (10.0 g, 32 mmol) and then suspended in 80 mL of DCM under argon atmosphere. Imidazole (4.4 g, 64 mmol) was then added as a solid and allowed to stir for 15 min. tert.butyldimethylsilyl chloride (7.3 g, 48.0 mmol) was then added as a solid and allowed to stir at room temperature for overnight or until completion of the starting material. Later, the reaction is quenched with 0.1M HCl and extracted with three times with dichloromethane and dried over $MgSO_4$. Solvents were removed *in vacuo*. Products were purified via SiO_2 gel flash column chromatography using hexanes and Ethyl acetate as eluent at 9:1 to afford intermediate-1 as a colorless liquid; Yield: 88%, 12.02 g.

Intermediate-2: To an oven dried two neck 100 mL round bottom flask taken $LiAlH_4$ (1.3 g, 33.8 mmol) slowly added 60 mL anhydrous THF at 0 ºC under argon atmosphere. Dissolved intermediate-1 in 20 mL solution and added slowly over 10 min to the above solution and stirred at room temperature for overnight or until completion of the starting material. On the next day cooled the reaction to 0 ºC and added 10% aq. NaOH solution (~10mL) dropwise with extra caution until no bubbles/gas were observed slowly for around 30 min and stirred for 30 min at room temperature. Filter the crude reaction by using celite and dried the filtrate with $MgSO_4$. Solvents were removed *in Vacuo*. Products were purified via SiO_2 gel flash column chromatography using hexanes and Ethyl acetate as eluent at 20:1 to afford intermediate-2 as a colorless liquid; Yield: 77%, 8.64 g.

Intermediate-3: To an oven dried two neck 100 mL round bottom flask taken intermediate-2 (8.64 g, 21.7 mmol) and added 40 mL anhydrous dichloromethane under argon atmosphere. Cooled the above solution to 0 ºC added DIPEA (9.5 mL, 54.2 mmol) slowly and added acryloyl chloride (2.1mL, 26.0 mmol) drop wise over 10 min and let it stir at room temperature for overnight or until completion of the starting material. The crude reaction is quenched with sat. $NaHCO_3$ and extracted with dichloromethane for 3 times. Organic layer is dried over anhydrous $MgSO_4$ and concentrated *in Vacuo*. Crude products were purified via SiO_2 gel flash column chromatography using hexanes and Ethyl acetate as eluent at 30:1 to afford intermediate-3 as a colorless liquid; Yield: 71%, 6.97 g.

Intermediate-4: To an oven dried 100-mL round-bottom flask equipped with a magnetic stir bar taken Intermediate-3 (6.97 g, 15.4 mmol) and then suspended in 60- mL of MeOH. 1% HCl was then added at 0 ºC dropwise and allowed to stir for overnight at room temperature or until completion of the starting material. The solvent is evaporated under reduced pressure and diluted with brine and extracted with dichloromethane for three times. Organic layer is dried over anhydrous $MgSO_4$ and concentrated *in Vacuo*. Crude products were purified via SiO_2 gel flash column chromatography using hexanes and Ethyl acetate as eluent at 40:1 to afford intermediate-4 as a colorless liquid; Yield: 63%, 3.3 g.

Synthesis of (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate:



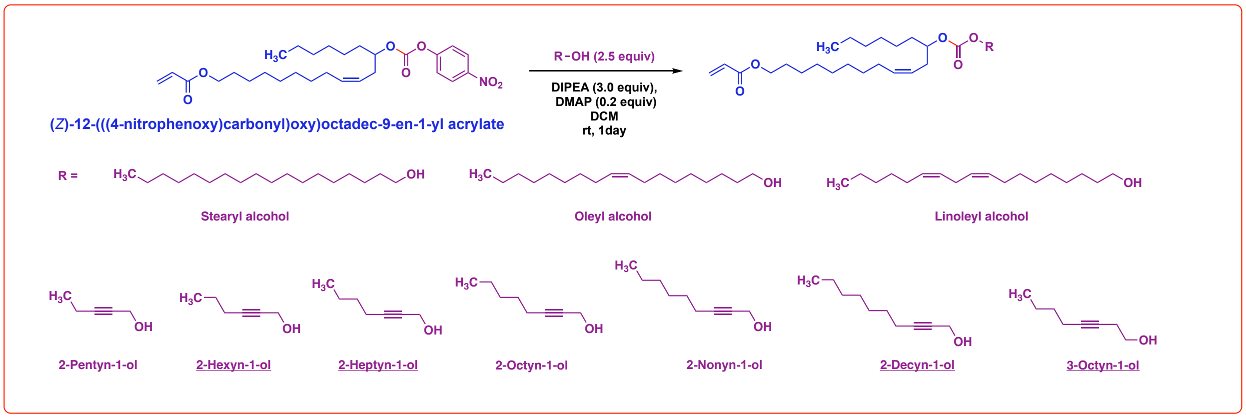
To a 100 mL round bottom flask taken 4-Nitrophenyl chloroformate (2.9 g, 14.6 mmol), and intermediate-4 (3.3 g, 9.7 mmol) and DCM (40 mL) is added under argon atmosphere. After dissolving all the starting materials slowly added pyridine (1.20 mL, 14.6 mmol) drop wise and let it stir at room temperature for overnight or until completion of the starting material. Reaction mass was diluted with brine (20 mL) and extracted with dichloromethane (3 X 100 mL) dried over anhydrous. $MgSO_4$ and concentrated under reduced pressure. Crude products were purified via SiO_2 gel flash column chromatography using hexanes and Ethyl acetate as eluent at 25:1 to afford (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate as a light yellow in color. Yield: 3.97 g, 81%.

^{1}H- NMR (500 MHz, $CDCl_3$): δ 8.29 (d, *J* = 9.0 Hz, 2H), 7.38 (d, *J* = 9.5 Hz, 2H), 6.40 (dd, *J1* = 17.5 Hz, *J2* = 1.5 Hz, 1H), 6.11 (dd, *J1* = 17.5 Hz, *J2* = 10.5 Hz, 1H), 5.82 (dd, *J1* = 10.0 Hz, *J2* = 1.5 Hz, 1H), 5.55 - 5.60 (m, 1H), 5.39 - 5.43 (m, 1H), 4.83 - 4.88 (m, 1H), 4.15 (t, *J* = 7.0 Hz, 2H), 2.40 - 2.53 (m, 2H), 2.06 – 2.10 (m, 2H), 1.65 - 1.75 (m, 4H), 1.27 - 1.45 (m, 18H), 0.90 (t, *J* = 7.0 Hz, 3H)

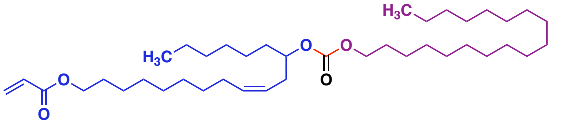
^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.3, 155.7, 152.3, 145.3, 133.7, 130.7, 130.5, 128.6, 125.3, 123.2, 121.8, 80.6, 64.7, 35.5, 31.9, 31.7, 29.5, 29.4, 29.23, 29.20, 29.1, 28.6, 27.4, 25.9, 25.2, 22.6, 14.1.

HRMS (m/z): [M+Na] + calcd for $C_{28}H_{41}NO_7Na$+ 526.2781, found: 526.2778; 0.57 ppm

General Procedure A, for synthesis of Carbonates of Ricinoleic acrylates:
Tails# 1-10



To a round bottom flask taken (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate (2.0 mmol, 1.0 equiv), DMAP (0.4 mmol, 0.2 equiv) and aliphatic alcohol (5.0 mmol, 2.5 equiv) in DCM (20 mL) is added. After dissolving all the starting materials slowly added DIPEA (6.0 mmol, 3.0 equiv) drop wise and let it stir at room temperature for overnight or until completion of the starting material. Reaction mass was diluted with brine (20 mL) and extracted with dichloromethane (3 X 100 mL). Organic layer is dried over anhydrous $MgSO_4$ and concentrated *in Vacuo*. Crude products were purified via SiO_2 gel flash column chromatography using hexanes and Ethyl acetate as eluent at 30:1 to afford Ricinoleic carbonates as a colorless liquid.

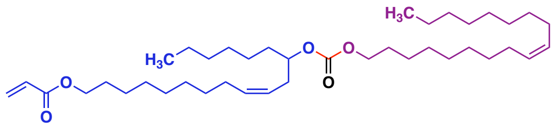


(Z)-12-(((octadecyloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate

Raj-2-105-1-AC1
RCB-tail#1

(Z)-12-(((octadecyloxy)carbonyl)oxy)octadic-9-en-1-yl acrylate (RCB-tail#1):
Prepared from general procedure **A** by using (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate and stearyl alcohol;
Yield: 975 mg, 77%.
^{1}H- NMR (500 MHz, $CDCl_3$): δ 6.40 (dd, *J1* = 17.0 Hz, *J2* = 1.5 Hz, 1H), 6.12 (dd, *J1* = 17.0 Hz, *J2* = 10.5 Hz, 1H), 5.82 (dd, *J1* = 10.0 Hz, *J2* = 1.5 Hz, 1H), 5.49 - 5.54 (m, 1H), 5.35 - 5.40 (m,

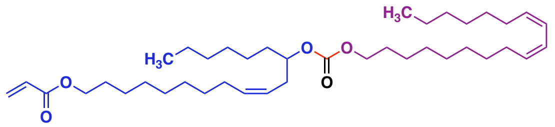
1H), 4.69 - 4.74 (m, 1H), 4.12 – 4.18 (m, 4H), 2.31 - 2.42 (m, 2H), 2.03 - 2.07 (m, 2H), 1.55 - 1.71 (m, 8H), 1.16 - 1.42 (m, 46H), 0.89 – 0.92 (m, 6H).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.4, 155.2, 132.0, 128.7, 123.8, 78.3, 67.9, 64.7, 33.6, 31.98, 31.94, 29.71, 29.69, 29.67, 29.6, 29.5, 29.43, 29.38, 29.3, 29.2, 29.1, 28.7, 28.6, 25.9, 25.7, 25.3, 22.7, 22.6, 14.13, 14.07.
HRMS (m/z): [M+Na] + calcd for $C_{40}H_{74}O_5$Na+, 657.5434 found: 657.5434; 0.0 ppm



(*Z*)-12-((((*Z*)-octadec-9-en-1-yl)oxy)carbonyl)oxy)octadec-9-en-1-yl acrylate

Raj-1-197-1-AC1
RCB-tail#2

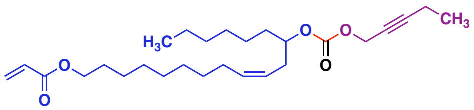
(*Z*)-12-(((((*Z*)-octadec-9-en-1-yl)oxy)carbonyl)oxy)octadec-9-en-1-yl acrylate (RCB-tail#2): Prepared from general procedure **A** by using (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate and oleyl alcohol;
Yield: 1060 mg, 84%.
^{1}H- NMR (500 MHz, $CDCl_3$): δ 6.39 (dd, *J1* = 17.0 Hz, *J2* = 1.5 Hz, 1H), 6.11 (dd, *J1* = 17.5 Hz, *J2* = 10.5 Hz, 1H), 5.82 (dd, *J1* = 10.5 Hz, *J2* = 1.5 Hz, 1H), 5.49 - 5.54 (m, 1H), 5.35 - 5.41 (m, 1H), 4.69 - 4.74 (m, 1H), 4.11 – 4.18 (m, 4H), 2.31 - 2.42 (m, 2H), 1.98 - 2.07 (m, 6H), 1.65 - 1.71 (m, 4H), 1.56 - 1.63 (m, 3H), 1.24 - 1.40 (m, 42H), 0.87 – 0.91 (m, 6H).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.3, 155.2, 132.0, 130.4, 130.0, 129.8, 128.7, 123.8, 78.3, 67.9, 64.7, 33.6, 32.0, 31.9, 31.7, 29.8, 29.7, 29.53, 29.52, 29.4, 29.33, 29.25, 29.2, 29.1, 28.7, 28.6, 25.9, 25.7, 22.7, 22.6, 14.12, 14.06
HRMS (m/z): [M+Na] + calcd for $C_{40}H_{72}O_5$Na+ 655.5277, found: 655.5272; 0.76 ppm



(*Z*)-12-((((9*Z*,11*Z*)-octadeca-9,11-dien-1-yl)oxy)carbonyl)oxy)octadec-9-en-1-yl acrylate

Raj-1-207-1-AC1
RCB-tail#3

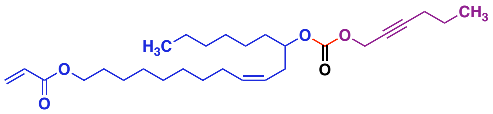
(*Z*)-12-(((((9*Z*,11*Z*)-octadeca-9,11-dien-1-yl)oxy)carbonyl)oxy)octadec-9-en-1-yl acrylate (RCB-tail#3):
Prepared from general procedure **A** by using (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate and Linoleyl alcohol;
Yield: 1044 mg, 83%.
^{1}H- NMR (500 MHz, $CDCl_3$): δ 6.40 (dd, *J1* = 17.5 Hz, *J2* = 1.5 Hz, 1H), 6.12 (dd, *J1* = 17.5 Hz, *J2* = 10.5 Hz, 1H), 5.82 (dd, *J1* = 10.0 Hz, *J2* = 1.5 Hz, 1H), 5.49 - 5.54 (m, 1H), 5.32 - 5.44 (m, 5H), 4.69 - 4.74 (m, 1H), 4.12 – 4.18 (m, 4H), 2.78 (t, *J* = 6.5 Hz, 2H), 2.31 - 2.42 (m, 2H), 2.02 - 2.09 (m, 6H), 1.65 - 1.71 (m, 4H), 1.56 - 1.63 (m, 3H), 1.24 - 1.41 (m, 35H), 0.88 – 0.93 (m, 6H).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.3, 155.2, 132.0, 130.4, 130.2, 130.1, 128.7, 128.0, 127.9, 123.8, 78.3, 67.9, 64.7, 33.6, 32.0, 31.9, 31.5, 30.0, 29.5, 29.43, 29.36, 29.2, 29.1, 28.7, 28.6, 27.4, 27.2, 25.9, 25.7, 25.6, 22.7, 22.6, 14.09, 14.07
HRMS (m/z): [M+Na] + calcd for $C_{40}H_{70}O_5$Na+ 653.5121, found: 653.5119; 0.31 ppm



(*Z*)-12-(((pent-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate

Raj-2-148-AC1
RCB-tail#4

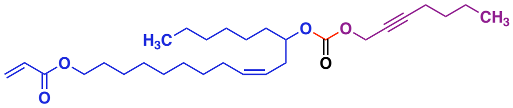
(*Z*)-12-(((pent-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate (RCB-tail#4): Prepared from general procedure **A** by using (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate and Pent-2-yn-1-ol alcohol;
Yield: 546 mg, 61%.
^{1}H- NMR (500 MHz, $CDCl_3$): δ 6.40 (dd, *J1* = 17.5 Hz, *J2* = 1.5 Hz, 1H), 6.11 (dd, *J1* = 17.5 Hz, *J2* = 10.5 Hz, 1H), 5.82 (dd, *J1* = 10.0 Hz, *J2* = 1.5 Hz, 1H), 5.49 - 5.54 (m, 1H), 5.34 - 5.40 (m, 5H), 4.70 - 4.75 (m, 3H), 4.16 (t, *J* = 6.5 Hz, 2H), 2.31 - 2.43 (m, 2H), 2.23 - 2.31 (m, 2H), 2.01 – 2.06 (m, 2H), 1.66 - 1.71 (m, 2H), 1.56 - 1.63 (m, 2H), 1.24 - 1.42 (m, 18H), 1.14 (t, *J* = 7.5 Hz, 3H) 0.88 (t, *J* = 7.0 Hz, 3H).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.4, 154.6, 133.1, 130.4, 138.7, 130.4, 133.1, 89.6, 79.0, 72.9, 64.7, 56.0, 33.6, 31.9, 31.7, 29.5, 29.4, 29.2, 29.1, 28.6, 27.3, 25.9, 25.2, 22.6, 14.1, 13.5, 12.5.
HRMS (m/z): [M+Na] + calcd for $C_{27}H_{44}O_5Na$+ 471.3086, found: 471.3084; 0.42 ppm



(*Z*)-12-(((hex-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate

Raj-2-145-AC1
RCB-tail#5

(*Z*)-12-(((hex-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate (RCB-tail#5): Prepared from general procedure **A** by using (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate and Hex-2-yn-1-ol alcohol;
Yield: 609 mg, 66%.
^{1}H- NMR (500 MHz, $CDCl_3$): δ 6.39 (dd, *J1* = 17.5 Hz, *J2* = 1.5 Hz, 1H), 6.11 (dd, *J1* = 17.5 Hz, *J2* = 10.5 Hz, 1H), 5.82 (dd, *J1* = 10.5 Hz, *J2* = 1.5 Hz, 1H), 5.48 - 5.53 (m, 1H), 5.34 - 5.39 (m, 1H), 4.69 - 4.76 (m, 3H), 4.15 (t, *J* = 6.5 Hz, 2H), 2.31 - 2.43 (m, 2H), 2.19 - 2.22 (m, 2H), 2.02 – 2.06 (m, 2H), 1.62 - 1.71 (m, 2H), 1.51 - 1.62 (m, 5H), 1.23 - 1.40 (m, 19H), 0.98 (t, *J* = 7.0 Hz, 3H) 0.88 (t, *J* = 7.0 Hz, 3H).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.4, 154.6, 133.1, 130.4, 128.7, 123.6, 88.2, 78.9, 73.7, 64.0, 55.9, 33.5, 31.9, 31.7, 29.5, 29.4, 29.2, 29.1, 28.6, 27.3, 25.9, 25.2, 22.6, 21.8, 14.1, 13.5.
HRMS (m/z): [M+Na] + calcd for $C_{28}H_{46}O_5Na$+ 485.3243, found: 485.3242; 0.21 ppm

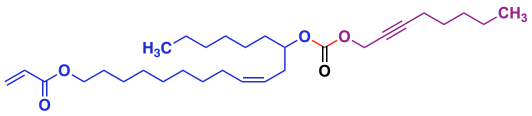


(*Z*)-12-(((hept-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate

Raj-2-149-AC1
RCB-tail#6

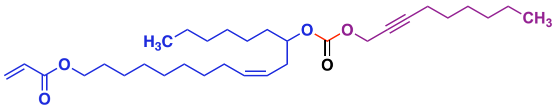
(*Z*)-12-(((hept-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate (RCB-tail#6):

Prepared from general procedure **A** by using (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate and Hept-2-yn-1-ol alcohol;
Yield: 618 mg, 65%.
^{1}H- NMR (500 MHz, $CDCl_3$): δ 6.39 (dd, *J1* = 17.5 Hz, *J2* = 1.5 Hz, 1H), 6.11 (dd, *J1* = 17.5 Hz, *J2* = 10.5 Hz, 1H), 5.82 (dd, *J1* = 10.5 Hz, *J2* = 1.5 Hz, 1H), 5.48 - 5.54 (m, 1H), 5.34 - 5.39 (m, 1H), 4.68 - 4.75 (m, 3H), 4.15 (t, *J* = 6.5 Hz, 2H), 2.31 - 2.43 (m, 2H), 2.22 - 2.35 (m, 2H), 2.02 – 2.07 (m, 2H), 1.58 - 1.71 (m, 4H), 1.48 - 1.54 (m, 2H), 1.23 - 1.40 (m, 20H), 0.88 – 0.93 (m, 6H).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.4, 154.6, 133.1, 130.4, 128.7, 123.6, 88.3, 78.9, 73.5, 64.7, 55.9, 33.5, 31.9, 31.7, 30.4, 29.5, 29.4, 29.2, 29.1, 28.6, 27.3, 25.9, 25.2, 22.6, 21.9, 18.4, 14.1, 13.5.
HRMS (m/z): [M+Na] + calcd for $C_{29}H_{48}O_5Na^+$ 499.3399, found: 499.3398; 0.20 ppm



(*Z*)-12-(((oct-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate
Raj-1-210-AC1
RCB-tail#7

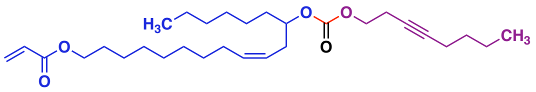
(*Z*)-12-(((oct-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate (RCB-tail#7):
Prepared from general procedure **A** by using (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate and Oct-2-yn-1-ol alcohol;
Yield: 753 mg, 77%.
^{1}H- NMR (500 MHz, $CDCl_3$): δ 6.40 (dd, *J1* = 17.0 Hz, *J2* = 1.5 Hz, 1H), 6.12 (dd, *J1* = 17.5 Hz, *J2* = 10.5 Hz, 1H), 5.82 (dd, *J1* = 10.5 Hz, *J2* = 1.5 Hz, 1H), 5.49 - 5.54 (m, 1H), 5.34 - 5.40 (m, 1H), 4.69 - 4.76 (m, 3H), 4.16 (t, *J* = 7.0 Hz, 2H), 2.31 - 2.43 (m, 2H), 2.21 - 2.24 (m, 2H), 2.02 – 2.07 (m, 2H), 1.69 – 1.72 (m, 2H), 1.58 - 1.64 (m, 2H), 1.50 - 1.56 (m, 2H), 1.23 - 1.42 (m, 21H), 0.88 – 0.93 (m, 6H).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.3, 154.6, 133.1, 130.4, 128.7, 123.7, 88.4, 79.0, 73.5, 64.7, 56.0, 33.5, 31.9, 31.7, 31.0, 29.5, 29.4, 29.2, 29.1, 28.6, 28.1, 27.4, 25.9, 25.2, 22.6, 22.2, 18.7, 14.1, 14.0.
HRMS (m/z): [M+Na] + calcd for $C_{30}H_{50}O_5Na^+$ 513.3556, found: 513.3551; 0.91 ppm



(*Z*)-12-(((non-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate
Raj-1-210-AC1
RCB-tail#8

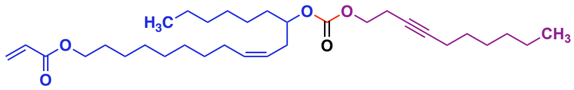
(*Z*)-12-(((non-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate (RCB-tail#8):
Prepared from general procedure **A** by using (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate and Non-2-yn-1-ol alcohol;
Yield: 845 mg, 84%.
^{1}H- NMR (500 MHz, $CDCl_3$): δ 6.40 (dd, *J1* = 17.0 Hz, *J2* = 1.5 Hz, 1H), 6.11 (dd, *J1* = 17.5 Hz, *J2* = 10.5 Hz, 1H), 5.82 (dd, *J1* = 10.5 Hz, *J2* = 1.5 Hz, 1H), 5.48 - 5.54 (m, 1H), 5.36 - 5.39 (m, 1H), 4.69 - 4.76 (m, 3H), 4.16 (t, *J* = 6.5 Hz, 2H), 2.31 - 2.43 (m, 2H), 2.21 - 2.31 (m, 2H), 2.02 – 2.07 (m, 2H), 1.66 – 1.71 (m, 2H), 1.58 - 1.63 (m, 2H), 1.49 - 1.56 (m, 2H), 1.24 - 1.42 (m, 24H), 0.88 – 0.92 (m, 6H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.4, 154.6, 133.1, 130.1, 130.4, 128.7, 123.7, 88.4, 79.0, 73.5, 64.7, 56.0, 33.5, 31.3, 29.5, 29.4, 29.2, 29.1, 28.6, 28.5, 28.3, 27.4, 25.9, 25.2, 22.6, 22.5, 18.8, 14.06, 14.05.
HRMS (m/z): [M+Na] + calcd for $C_{31}H_{52}O_5Na+$ 527.3712, found: 527.3711; 0.19 ppm



(*Z*)-12-(((oct-3-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate
Raj-2-98-AC1
RCB-tail#9

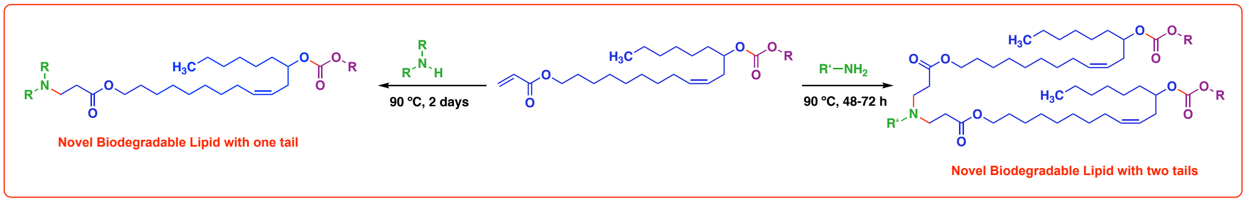
(*Z*)-12-(((oct-3-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate (RCB-tail#9):
Prepared from general procedure **A** by using (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate and Oct-3-yn-1-ol alcohol;
Yield: 793 mg, 81%.
^{1}H- NMR (500 MHz, $CDCl_3$): δ 6.40 (dd, *J1* = 17.5 Hz, *J2* = 1.5 Hz, 1H), 6.12 (dd, *J1* = 17.5 Hz, *J2* = 10.0 Hz, 1H), 5.82 (dd, *J1* = 10.5 Hz, *J2* = 1.5 Hz, 1H), 5.49 - 5.54 (m, 1H), 5.34 - 5.39 (m, 1H), 4.69 - 4.76 (m, 3H), 4.16 – 4.21 (m, 4H), 2.52 - 2.59 (m, 2H), 2.31 - 2.43 (m, 2H), 2.14 – 2.18 (m, 2H), 2.02 – 2.07 (m, 2H), 1.63 – 1.71 (m, 2H), 1.56 - 1.60 (m, 2H), 1.25 - 1.51 (m, 21H), 0.88 – 0.93 (m, 6H).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.4, 154.8, 133.1, 130.1,130.4, 128.7, 123.7, 82.3, 78.6, 74.9, 65.9, 64.7, 33.6, 31.9, 31.7, 29.5, 29.4, 29.3, 29.2, 29.1, 28.6, 27.4, 25.9, 25.3, 22.6, 18.4, 14.1, 13.6.
HRMS (m/z): [M+Na] + calcd for $C_{30}H_{50}O_5Na+$ 513.3556, found: 513.3550; 1.17 ppm

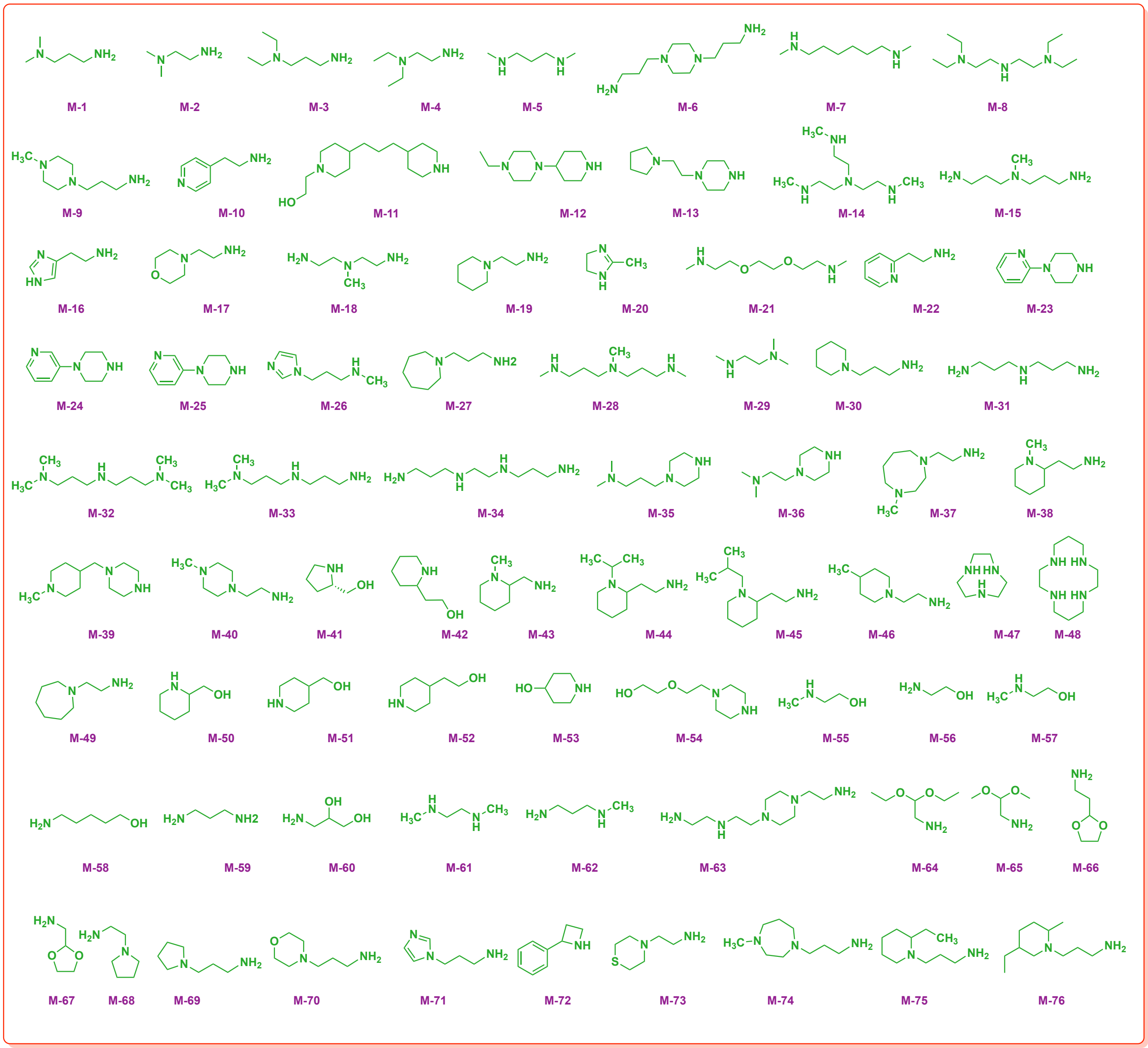


(*Z*)-12-(((dec-3-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate
Raj-2-146-AC1
RCB-tail#10

(*Z*)-12-(((dec-3-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate (RCB-tail#10):
Prepared from general procedure **A** by using (Z)-12-(((4-nitrophenoxy)carbonyl)oxy)octadic-9-en-1-yl acrylate and Dec-3-yn-1-ol alcohol;
Yield: 848 mg, 82%.
^{1}H- NMR (500 MHz, $CDCl_3$): δ 6.40 (dd, *J1* = 17.0 Hz, *J2* = 1.5 Hz, 1H), 6.11 (dd, *J1* = 17.5 Hz, *J2* = 10.0 Hz, 1H), 5.82 (dd, *J1* = 10.5 Hz, *J2* = 1.5 Hz, 1H), 5.48 - 5.53 (m, 1H), 5.34 - 5.40 (m, 1H), 4.69 - 4.74 (m, 3H), 4.15 – 4.20 (m, 4H), 2.53 - 2.56 (m, 2H), 2.32 - 2.42 (m, 2H), 2.13 – 2.15 (m, 2H), 2.02 – 2.06 (m, 2H), 1.58 – 1.71 (m, 2H), 1.45 - 1.51 (m, 2H), 1.25 - 1.40 (m, 24H), 0.88 – 0.92 (m, 6H).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.3, 154.8, 133.1, 130.4, 128.7, 123.7, 82.3, 78.6, 74.9, 65.9, 64.7, 33.6, 31.9, 31.7, 31.4, 29.5, 29.4, 29.2, 29.1, 28.8, 28.6, 28.5, 27.3, 25.9, 25.2, 22.57, 22.56, 19.4, 18.7, 14.1.
HRMS (m/z): [M+Na] + calcd for $C_{32}H_{54}O_5Na+$ 541.3869, found: 541.3869; 0.0 ppm

General Procedure B, for Michael addition reaction with carbonate derived Ricinoleic Acrylate:

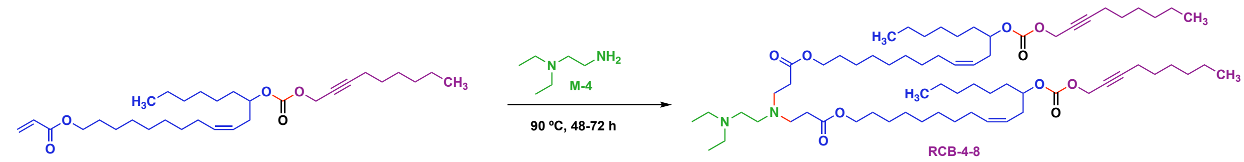




Into a 1-dram scintillation vial equipped with magnetic stir bar was placed carbonate derived Ricinoleic acrylate (0.5 mmol, 2.5 equiv) and Aliphatic amine (0.2 mmol, 1.0 equiv). Generally, the lipids are synthesized with a molar ratio of amine/Ricinoleic acrylate carbonates in 1:2.5 and 1:1.25 equiv for primary and secondary amines respectively. The vial was sealed with a silicone-lined screwcap and stirred at 90 ºC for 2-3 days or until complete conversion of the starting material amine. Further purification is achieved by flash column chromatography on an Isco Combiflash system eluting with a gradient (0-100%) Ultra (3% concentrated ammonium hydroxide, 22%

methanol, 75% dichloromethane): dichloromethane to afford carbonate derived Ricinoleic acrylate based ionizable lipids (RCB 01 to RCB 720).

Synthesis of bis((Z)-12-(((non-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl) 3,3'-((2-(diethylamino)ethyl)azanediyl)dipropionate:



(Z)-12-(((non-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate

bis((Z)-12-(((non-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl) 3,3'-((2-(diethylamino)ethyl)azanediyl)dipropionate

Into a 1-dram scintillation vial equipped with magnetic stir bar was placed (*Z*)-12-(((non-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl acrylate (252 mg, 0.5 mmol, 2.5 equiv) and *N*,*N*-diethylethane-1,2-diamine (23.2 mg, 0.2 mmol, 1.0 equiv). The vial was sealed with a silicone-lined screwcap wrapped the vial cap with electrical tape and stirred at 90 ºC for 2-3 days or until complete conversion of the starting material amine. Further purification is achieved by flash column chromatography on an Isco Combiflash system eluting with a gradient (0-100%) Ultra (3% concentrated ammonium hydroxide, 22% methanol, 75% dichloromethane): dichloromethane to afford bis((Z)-12-(((non-2-yn-1-yloxy)carbonyl)oxy)octadec-9-en-1-yl)3,3'-((2-(diethylamino)ethyl)azanediyl)dipropionate as a colorless oil (Yield: 173 mg, 77%)

^{1}H- NMR (500 MHz, $CDCl_3$): δ 5.48 - 5.54 (m, 2H), 5.34 - 5.39 (m, 2H), 4.68 - 4.76 (m, 6H), 4.06 (t, *J* = 7.0 Hz, 4H), 2.80 (t, *J* = 7.5 Hz, 4H), 2.52 - 2.57 (m, 8H), 2.45 (t, J = 7.5 Hz, 4H), 2.36 – 2.42 (m, 4H), 2.21 - 2.25 (m, 4H), 2.02 - 2.07 (m, 4H), 1.63 – 1.67 (m, 1H), 1.49 – 1.58 (m, 4H), 1.24 - 1.42 (m, 45H), 1.03 (t, *J* = 7.0 Hz, 6H), 0.88 (m, 12H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ 172.7, 154.6, 133.1, 123.7, 88.4, 79.0, 73.5, 64.6, 56.0, 52.2, 51.3, 49.8, 47.5, 33.5, 32.7, 31.9, 31.7, 31.3, 29.54, 29.48, 29.3, 29.1, 28.5, 28.3, 27.4, 25.9, 25.2, 22.6, 22.5, 18.8, 14.07, 14.05, 11.8.

HRMS (m/z): [M+H] + calcd for $C_{68}H_{121}N_2O_{10}^+$ 1125.9021, found: 1125.9018; 0.27 ppm

Quantification of lipids by QToF-LCMS. Lung samples were homogenized following the addition of 19 equivalent (eq.) (w/v) of ethanol (dilution factor [DF] = 20), and protein was precipitated and analyzed against calibration standards prepared in matching blank. A QToF-LCMS system was used for signal detection.

Code used for deep sequencing data analysis.

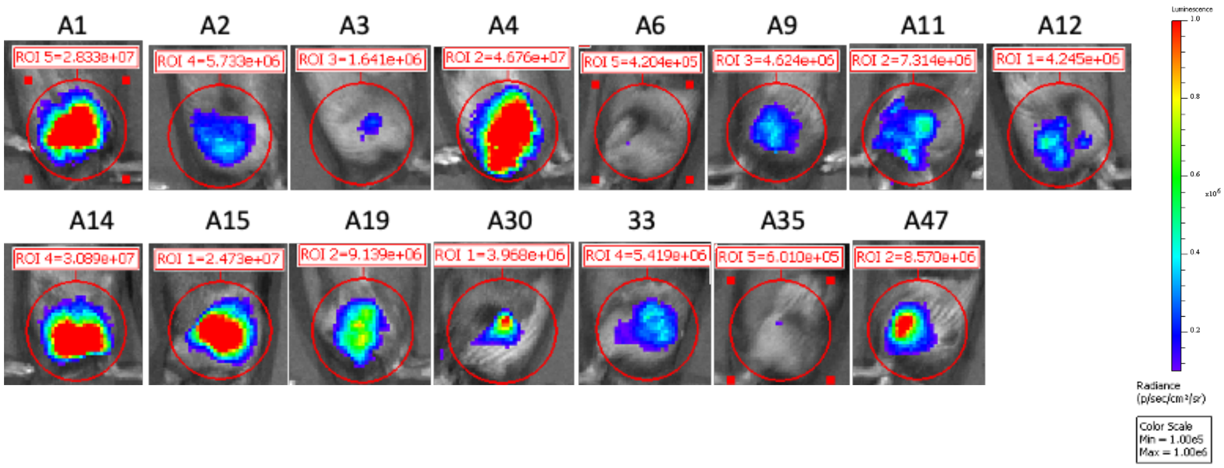
```
CRISPRessoBatch --batch_settings sgA.batch --amplicon_seq
atcaagctgatccggaacccttaatataacttcgtataatgtatgctatacgaagttattaggtccctcgacctgcagcccaagctagatcgaat
tcggccgg -g gtatgctatacgaagttatt --max_paired_end_reads_overlap 200 -w 10 -q 30  --
write_detailed_allele_table --write_cleaned_report --place_report_in_output_folder --
min_frequency_alleles_around_cut_to_plot 0.001 --plot_window_size 35

CRISPRessoBatch --batch_settings sgB.batch --amplicon_seq
GGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGAT
GCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAAT
TGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAG
TAAAACCTCTACAAATGTGGTATGGCTGATTATGATCCTCTAGAGTCGCA -g
AAGTAAAACCTCTACAAATG --max_paired_end_reads_overlap 200 -w 10 -q 30  --
write_detailed_allele_table --write_cleaned_report --place_report_in_output_folder --
min_frequency_alleles_around_cut_to_plot 0.001 --plot_window_size 35
```

sgRNA Ai9: aagtaaaacctctacaaatg
sgRNA A: aaagaattgatttgataccg
sgRNA B: gtatgctatacgaagttatt

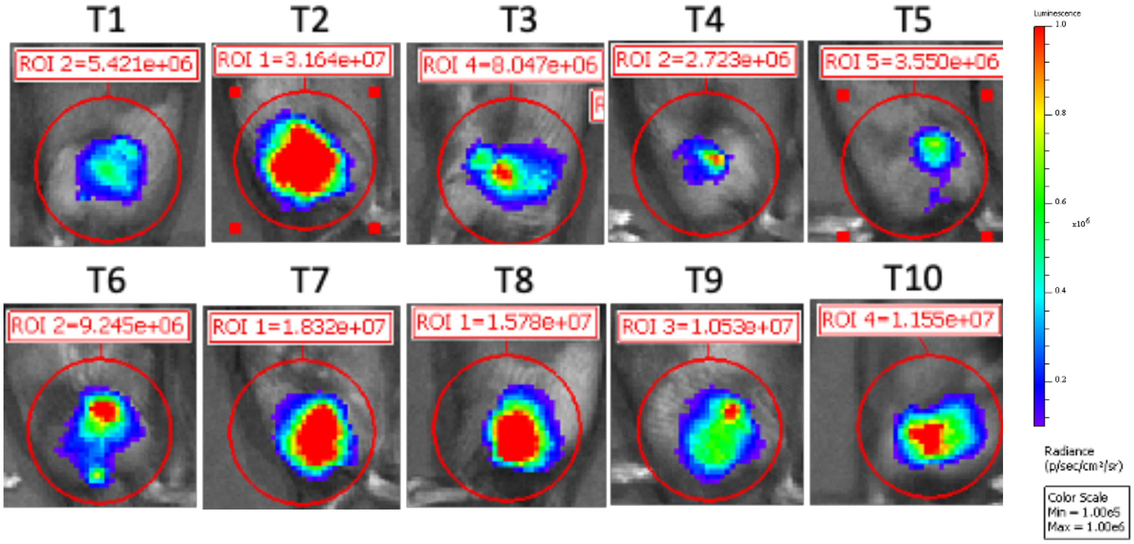
Primers for deep sequencing:

sgA_deepF	ctacacgacgctcttccgatctGGGCAACGTGCTGGTTATTG	For sgRNA A
sgA_deepR	agacgtgtgctcttccgatctTCGCGAATAACTTCGTATAGCA	For sgRNA A
sgB_deepF	ctacacgacgctcttccgatctATCAAGCTGATCCGGAACCC	For sgRNA B
sgB_deepR	agacgtgtgctcttccgatctCCGGCCGAATTCGATCTAGC	For sgRNA B

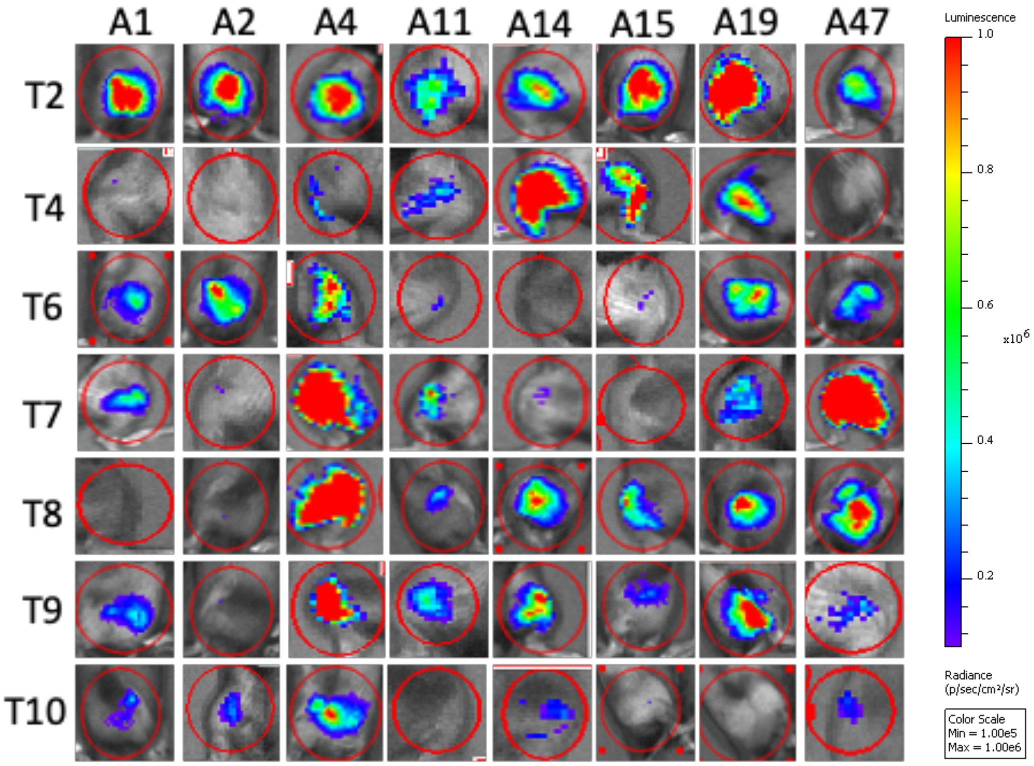
Supplementary Figures



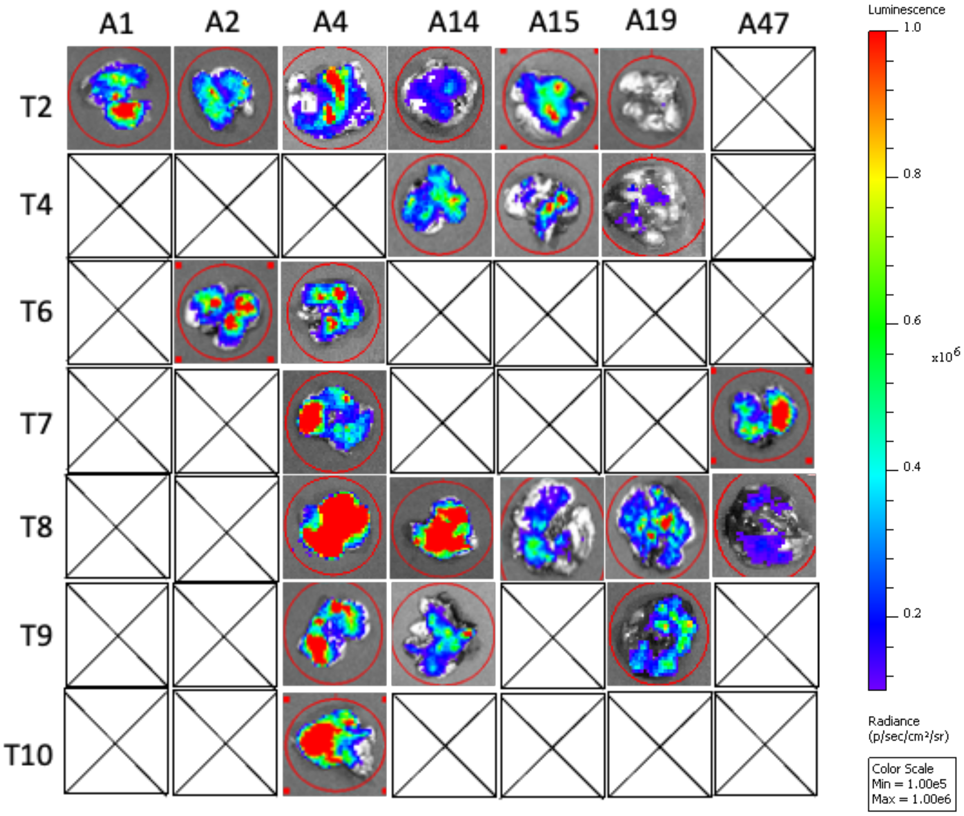
Supplementary Figure S1. Representative IVIS images of the first-round *in vivo* batch-based testing (intramuscular injection, 0.5 mg/kg mLuc, 10 LNP mixtures per mouse) from two mice per group.



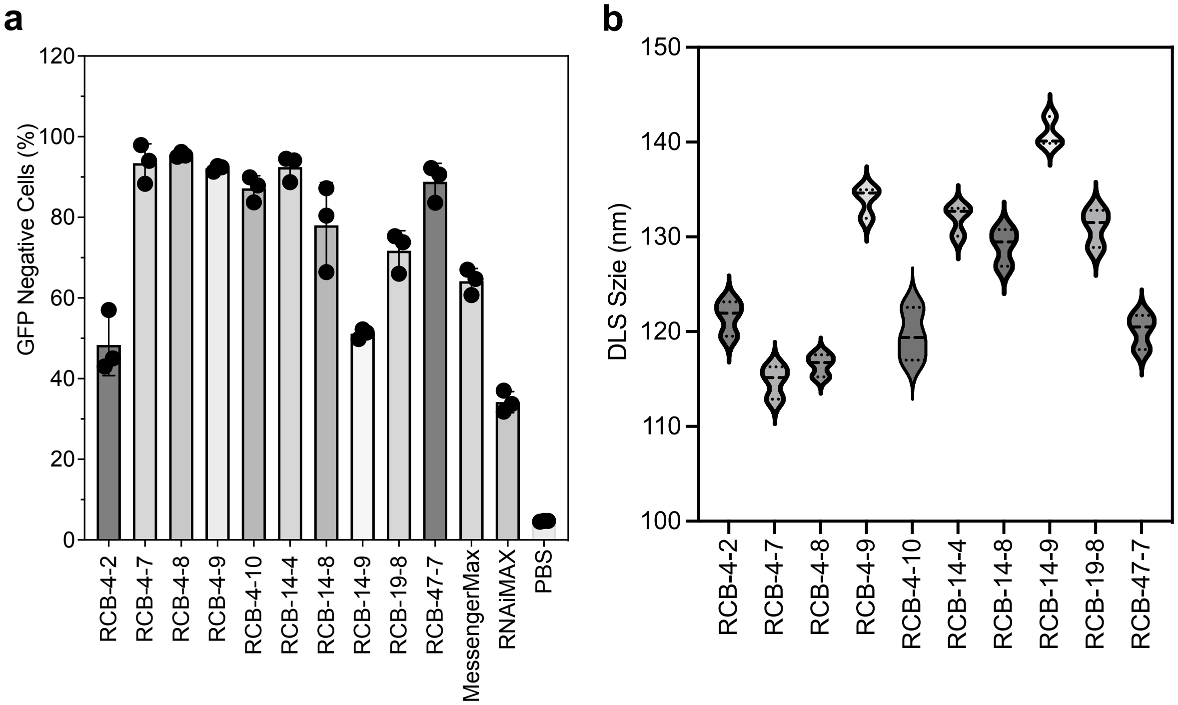
Supplementary Figure S2. Representative IVIS images of the second-round *in vivo* batch-based testing (intramuscular injection, 0.4 mg/kg, 8 LNP mixtures per mouse) from two mice per group.



Supplementary Figure S3. Representative IVIS images of the third-round *in vivo* testing (intramuscular injection, 0.25 mg/kg mLuc per mouse) from two mice per group.

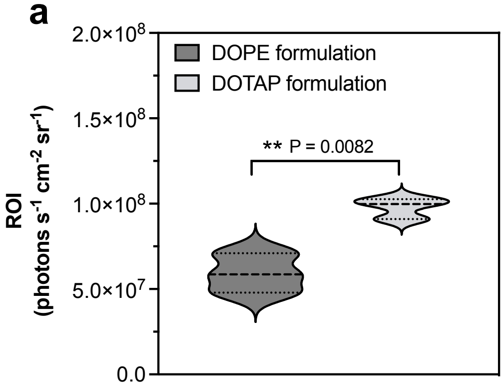


Supplementary Figure S4. Representative IVIS images of the fourth-round *in vivo* testing (intratracheal administration, 0.25 mg/kg mLuc per mouse) from two mice per group.



Supplementary Figure S5. *In vitro* CRISPR-Cas9 knockout assay. a, nine top-performing LNPs formulated with CRISPR-Cas9 mRNA/GFP-sgRNA were added to the green fluorescent protein (GFP)-expressing HEK cells. After 72h incubation, the percentage of GFP-negative HEK cells was analyzed by flow cytometry. Results were obtained from three biologically independent samples and presented as mean ± SD. **b,** DLS sizes of nine LNPs encapsulated with CRISPR-Cas9 mRNA/GFP-sgRNA; LNPs were prepared by directing mixing with pipettes. Results were obtained from three independent experiments.

a



b

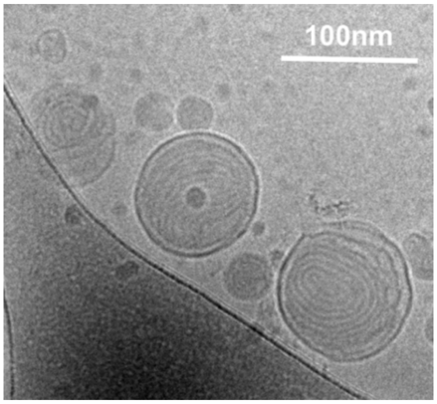
Pipette mixing

Size (nm)	PDI	Zeta potential	EE (%)
112.9±5.1	0.20±0.03	10.8±0.9	78.4±5.8

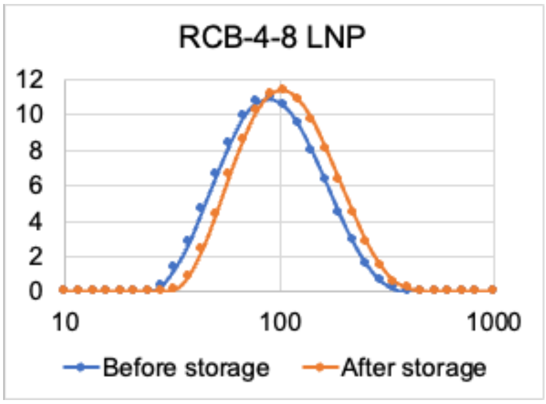
Microfluidic mixing

Size (nm)	PDI	Zeta potential	EE (%)
85.7±1.6	0.11±0.02	9.7±0.6	87.1±2.3

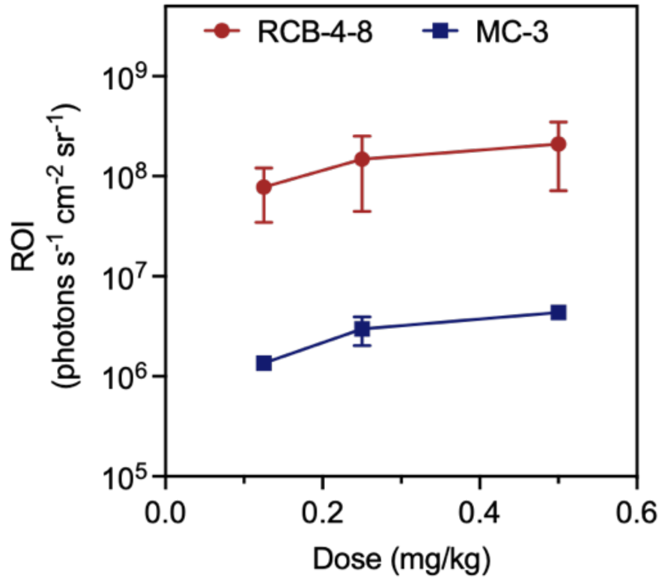
c



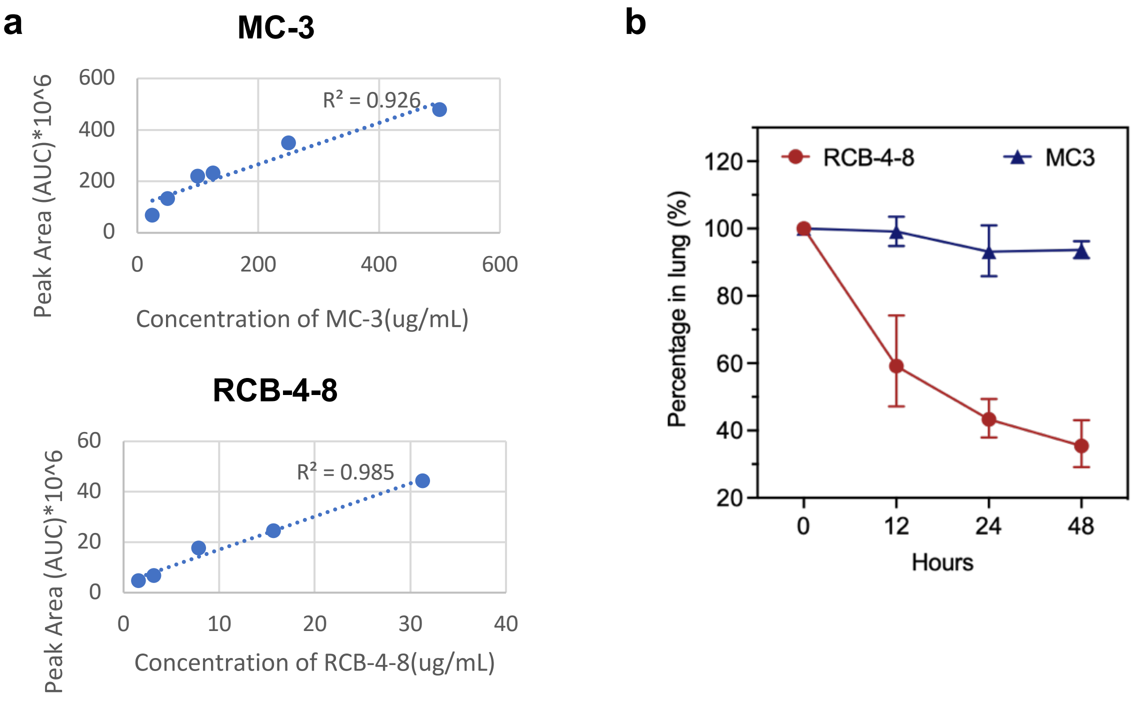
d



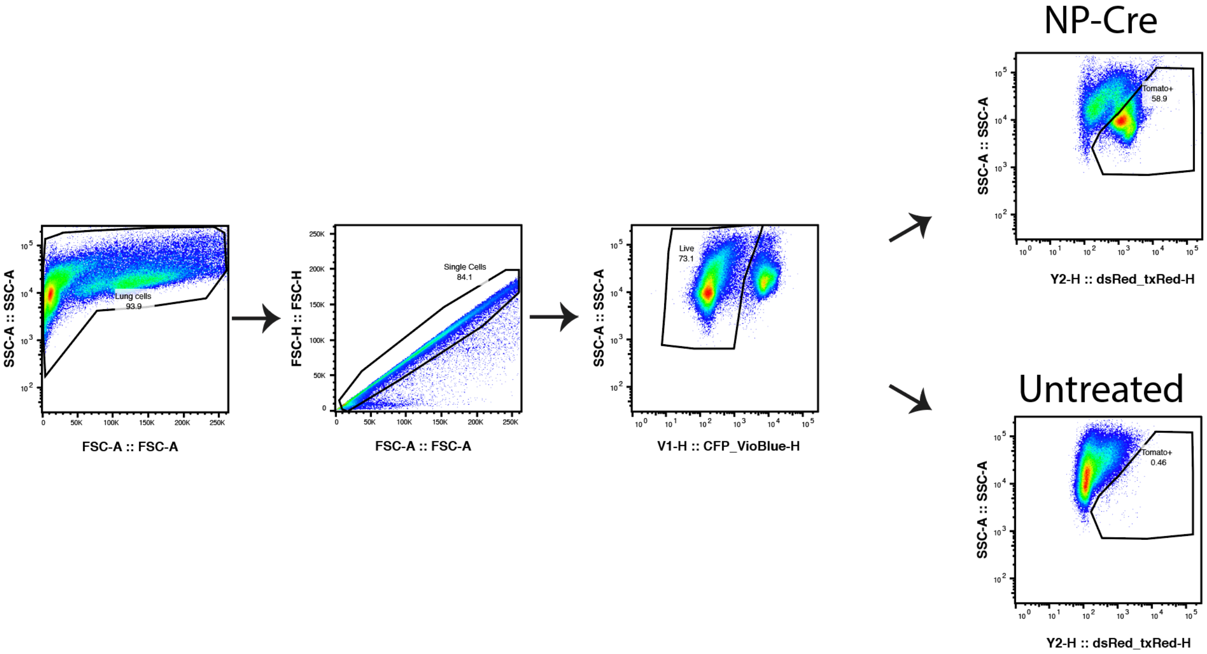
Supplementary Figure S6. Characterization of RCB-4-8 LNP. a, Pulmonary transfection efficiency of RCB-4-8 LNPs formulated with either DOPE or DOTAP. Results were obtained from three mice per group. Total flux of Luciferase signal (ROI) was measured at 6 hours following intratracheal administration of mLuc LNPs (0.125mg/kg). Statistical comparison was made using unpaired two-tailed Student's t-test. **P<0.01. **b,** Characterization of RCB-4-8 LNPs encapsulating firefly luciferase mRNA made by pipette or microfluidic mixing. **c,** Representative Cryo-TEM image of RCB-4-8 LNPs from three independent samples. **d**, Size of RCB-4-8 LNPs measured by dynamic light scattering before and after storage at -20°C for 1 month. 10% sucrose was added to the RCB-4-8 LNP solution before the stability test.



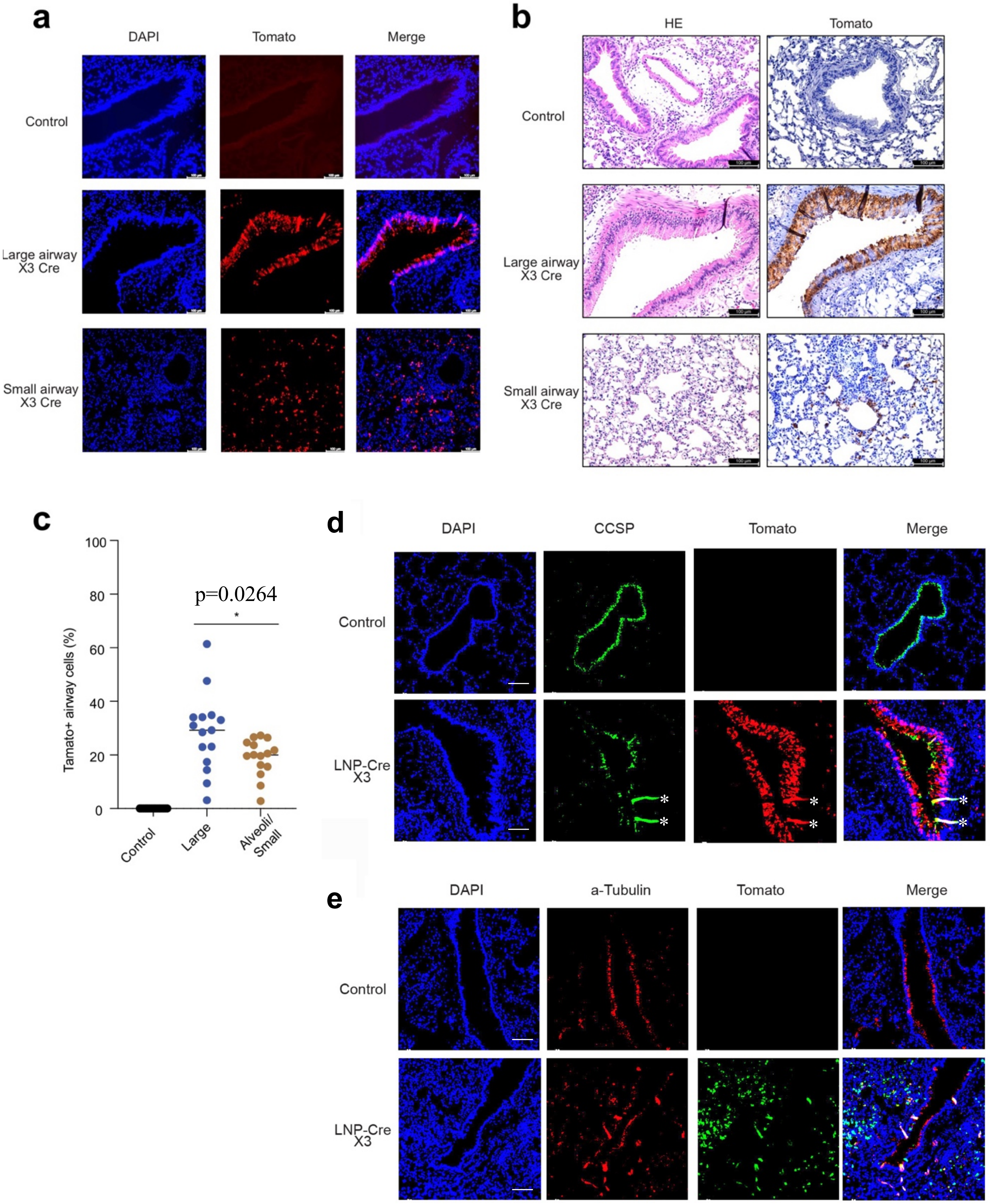
Supplementary Figure S7. Dose-response curves of MC3 and RCB-4-8 mLuc LNPs. Total flux of Luciferase signal (ROI) was measured at 6 hour following intratracheal administration of mLuc LNPs at different dosages. Results were obtained from three mice per group and presented as mean ± SD.

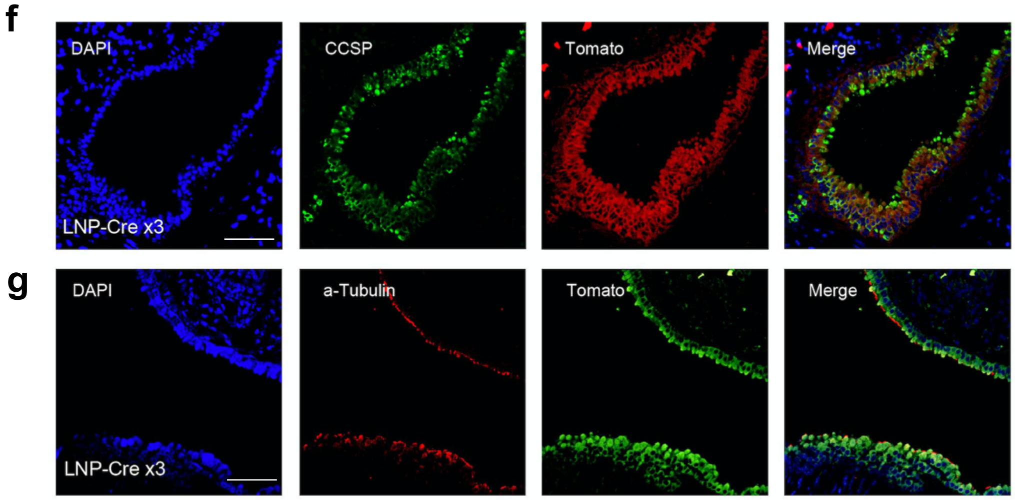


Supplementary Figure S8. a, Standard curves of MC3 and RCB-4-8. The standard curves for the lipids MC-3 (concentration ranging from 25 µg to 500 µg, $R^2 = 0.93$) and RCB-4-8 (concentration ranging from 1 µg to 30 µg, $R^2 = 0.98$) were drawn for the quantification of residual lipids. **b, Elimination kinetics in the lung.** Clearance curves of RCB-4-8 and MC3 in the lung within 48 hr following intratracheal administration. Results were obtained from three mice per group and presented as mean ± SD.

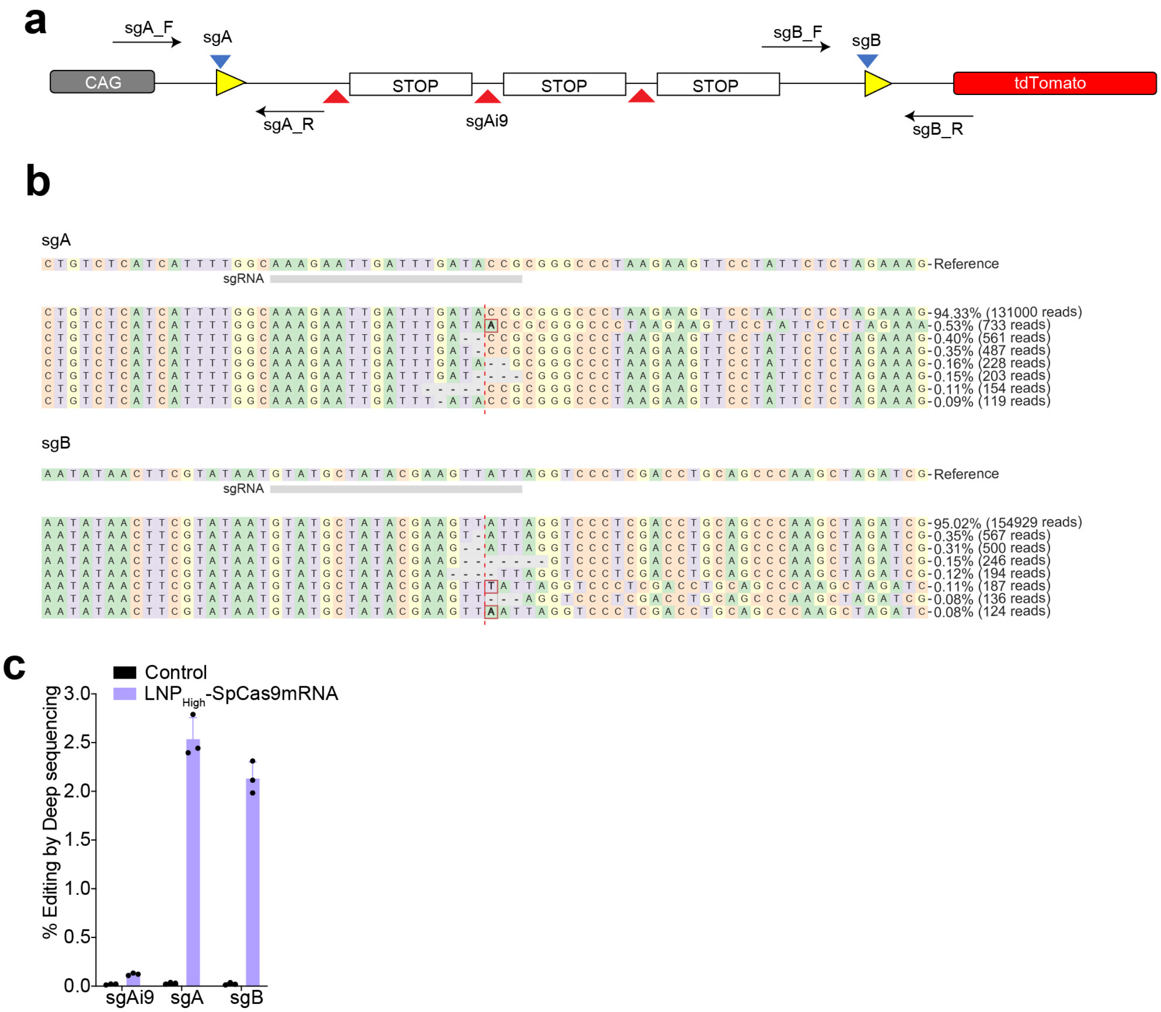


Supplementary Figure S9. Flow cytometry gating strategy. Single cells prepared from Ai9 mouse lungs were gated. Viable cells (VioBlue negative) expressing tdTomato fluorescence (txRed positive) were analyzed by flow cytometry (n = 3).


a
DAPI
Tomato
Merge
Control
Large airway X3 Cre
Small airway X3 Cre
b
HE
Tomato
Control
Large airway X3 Cre
Small airway X3 Cre
c
Tamato+ airway cells (%)
100
80
60
40
20
0
p=0.0264
*
Control
Large
Alveoli/ Small
d
DAPI
CCSP
Tomato
Merge
Control
LNP-Cre X3
*
*
e
DAPI
a-Tubulin
Tomato
Merge
Control
LNP-Cre X3



Supplementary Figure S10. Genome editing in mouse lung with RCB-4-8 LNP-Cre-mRNA. **a**, Ai9 mice were dosed with three sequential deliveries (over four days) of Cre-mRNA. Three days after the last dose, the lungs were collected. Representative native fluorescence images of lung sections are shown. Results were obtained from 3 independent experiments. Saline serves as a negative control. Scale bar: 100µm. **b**, Paraffin-embedded lung sections were stained with antibodies for tdTomato (red). **c,** Quantification of IHC in **b**. Results were obtained from three mice per group (n=5 sections per mouse.) and presented as mean ± SD. *$P<0.05$ by one-way ANOVA with Tukey's multiple comparisons test. **d, f,** Paraffin-embedded lung sections (n=3) were stained with antibodies for CCSP (green), a marker of club/Clara cells, and tdTomato (red). *denote autofluorescence. Scale bar: 100µm. **e, g,** Lung sections (n=3) were stained with antibodies for acetylated tubulin (red), a marker of ciliated cells, and tdTomato (green). Scale bar: 100µm. **d** and **e** were collected by a Leica DMi8 imaging microscope, while **f** and **g** were collected by a ZEISS confocal microscope (40X oil lens). Results were obtained from 3 independent experiments.



Supplementary Figure S11. Indel rates at Ai9 locus in mice lung. **a**, PCR primers used for targeted amplicon deep sequencing. **b-c**, Frequencies of editing were quantified by deep sequencing from PCR amplicons spanning each locus. Results were obtained from three mice per group and presented as mean ± SD.