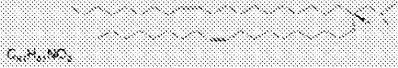


Figure 1

An Exemplary Composition of the LNP-1 Lipid Nanoparticle Platform: Structural Lipid Components

Lipid Components (LNP1)	Lipid Component & Formula	Mw (g/mol)	Exact Mass (g/mol)
DOOMA (18:1)	 $C_{60}H_{101}NO_9$	620.10	620.63
L1A (18:1)	 $C_{60}H_{101}NO_9P$	~2839.57 (Average)	~2837.79 (Average)
DSPE (18:0)	 $C_{60}H_{101}NO_9P$	790.16	789.62
Cholesterol	 $C_{27}H_{46}O$	386.65	386.35

Drug Product Formulation Buffer Solution pH 7.4 ± 0.2 , -18.7 ± 0.8 mV at 25°C (Exemplary):

- Sodium Phosphate Dibasic: 10.14 mM
- Potassium Phosphate Monobasic: 1.76 mM
- Potassium Chloride: 2.7 mM
- Sodium Chloride: 137 mM
- Nuclease-Free Water, not DEPC-Treated

Figure 2

Lipid Composition of the LNP-1 Platform (Exemplary)

Sample	Lipid Name	Calculated Concentration (mg/ml)	LNP Total Mass (mg/ml)	MW	mmol in 1ml	Total Count (mmol)	Assay mmol%	Assay m/m%
eGFP mRNA/LNP-1 (Freshly-formulated)	DODMA	3.254	6.448	620.10	0.005830	0.001256	46.41	50.46
	DSPE	1.024		790.15	0.001440		11.46	15.89
	Cholesterol	1.802		385.65	0.005191		41.33	27.94
	L1A	0.245		2841.00	0.000096		0.76	3.80

Identity of LNP-1 Formulations: Four Lipid Components
Separation of Four Lipid Components of LNP-1 Formulations Obtained by UPLC-ELSD Method

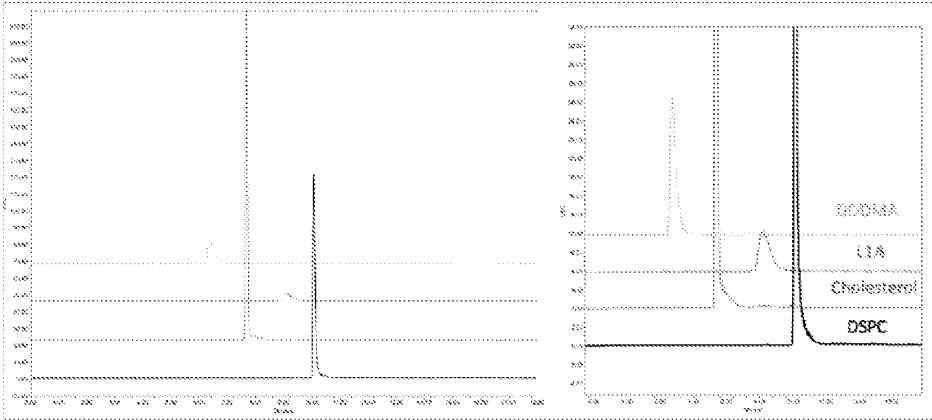


Figure 3

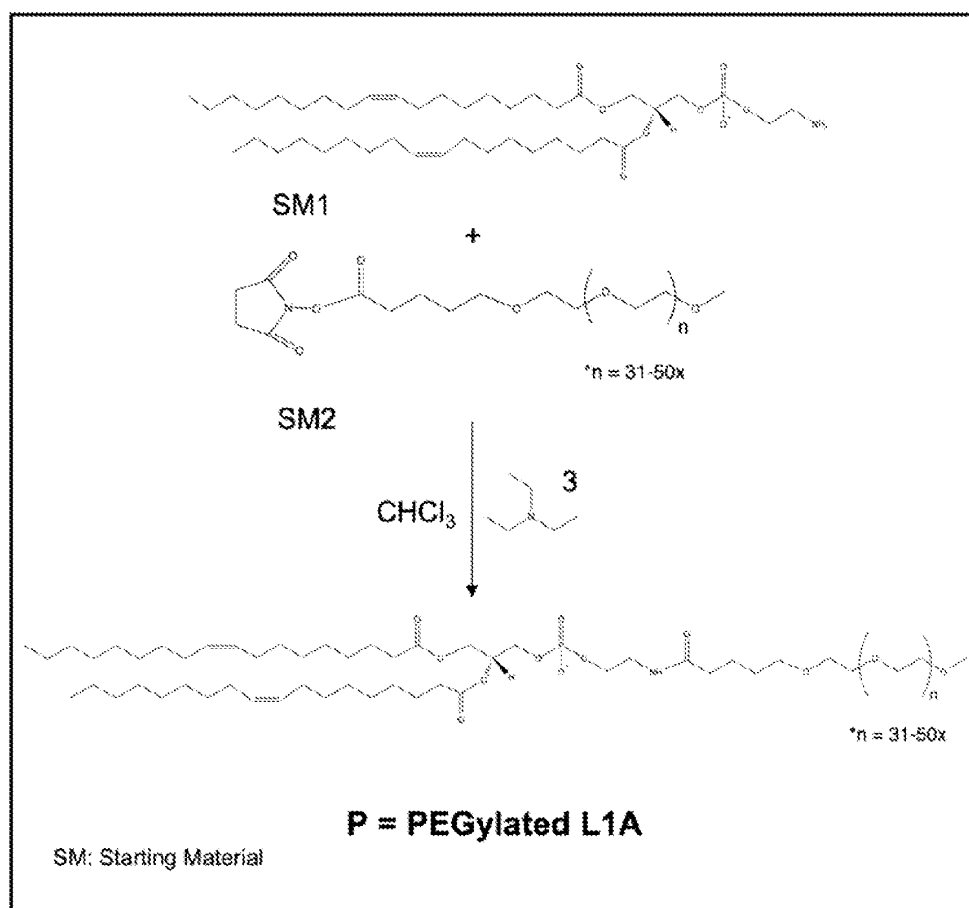
Synthesis of PEGylated L1A (Exemplary)

Figure 3 (Cont.)

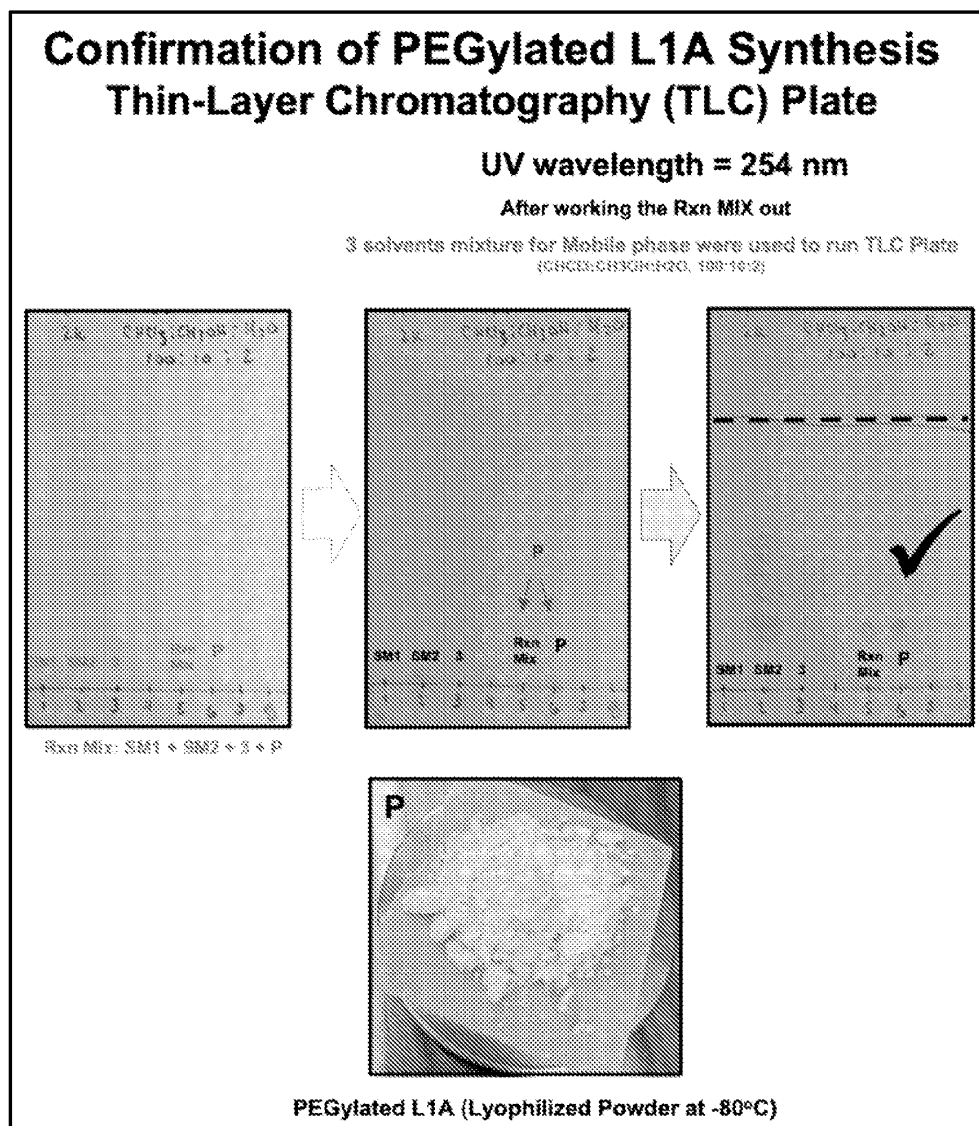


Figure 3 (Cont.)

Identity, Purity and Integrity Confirmation of PEGylated L1A
PEGylated L1A Successfully Identified, Integrity Confirmed with No Impurity
Identity of PEGylated L1A: Proton ^1H NMR

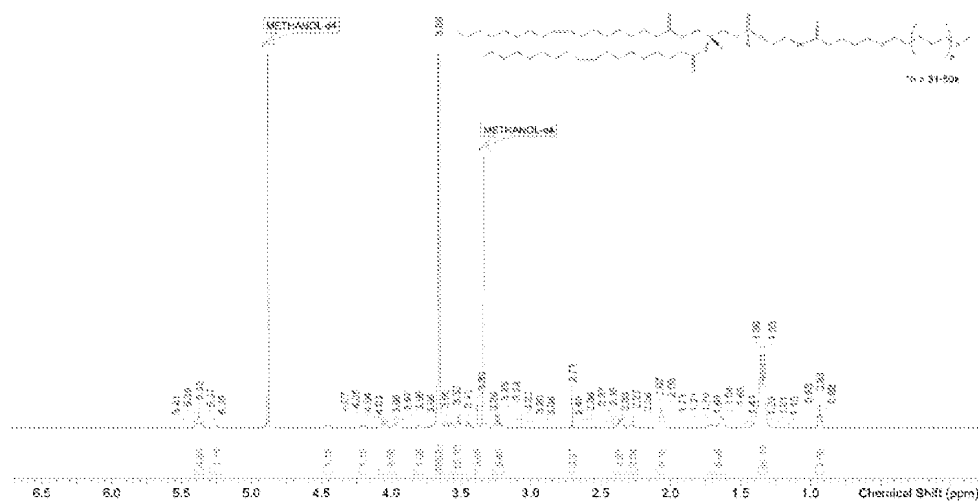


Figure 3 (Cont.)

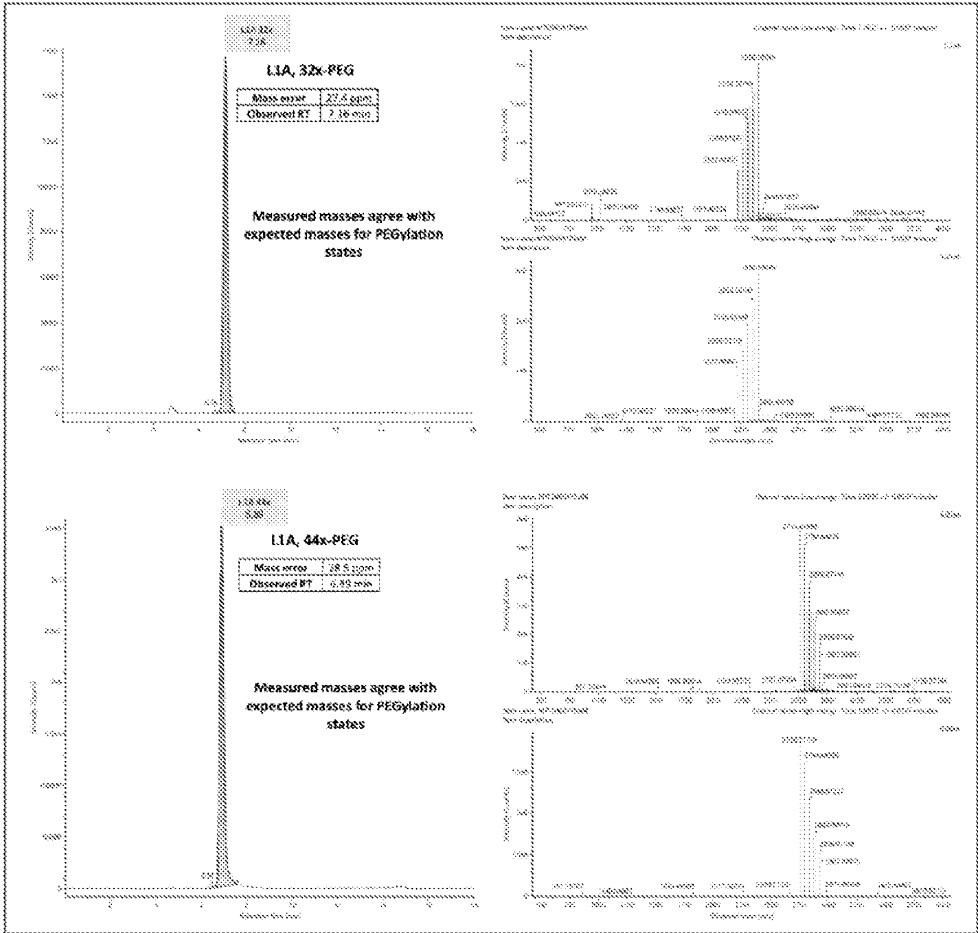


Figure 3 (Cont.)

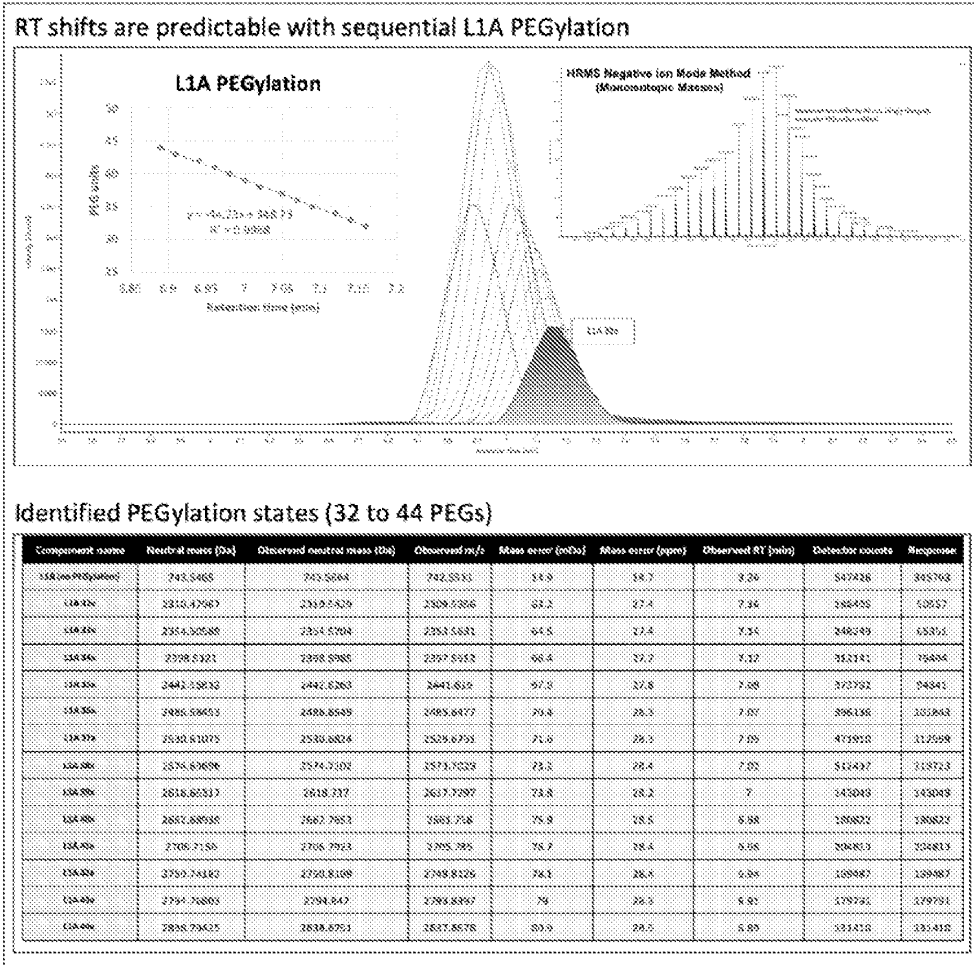
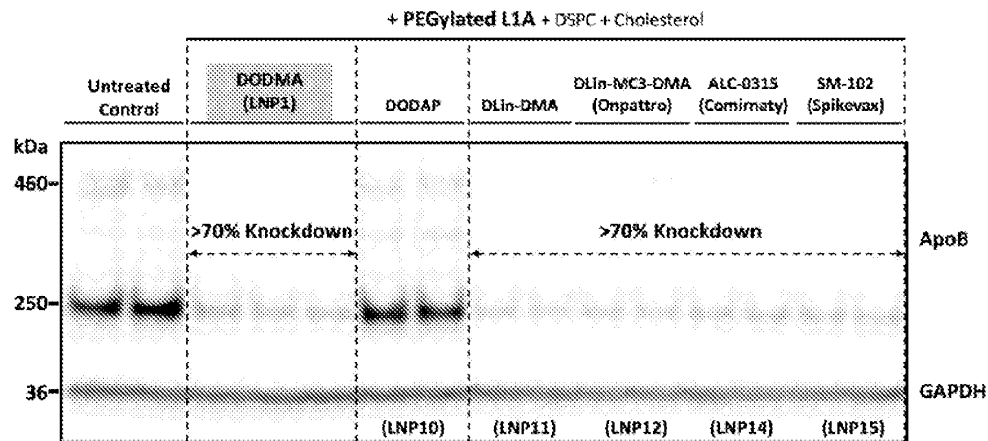


Figure 4

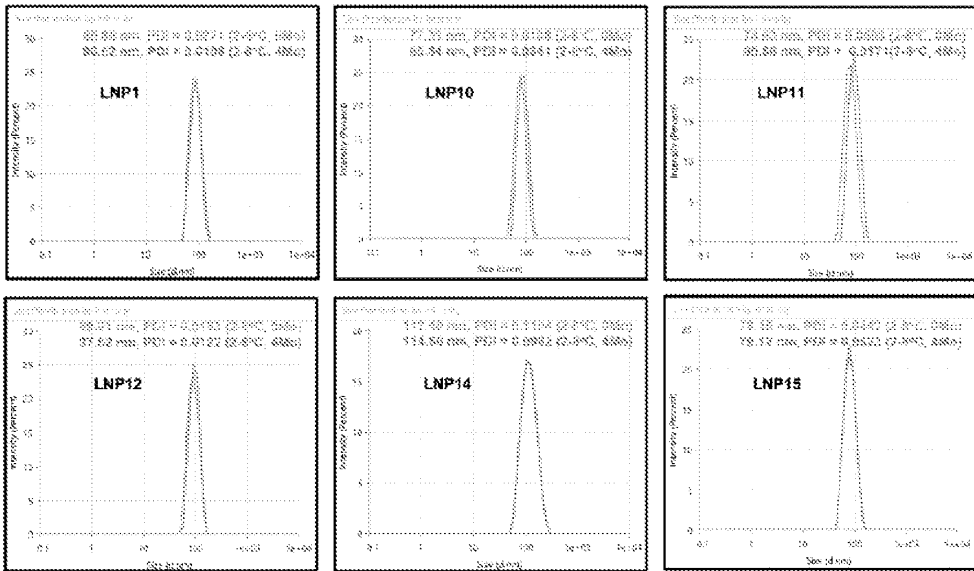
High Compatibility of PEG-conjugated Phospholipid of LNP-1 (Exemplary)

LNP-1's Flexibility with Structural Ionizable Lipid Component (Exemplary)



LNP ID	IL Lipid Component	IL Lipid Structure & Formula	+ PEGylated L1A + DSPC + Cholesterol	Mw (g/mol)	Exact Mass (g/mol)
LNP1	DODMA 18:1	 $C_{42}H_{82}NO_6$	L1A $C_{42}H_{82}NO_6P$	620.10	620.63
LNP10	DODAP 18:1	 $C_{42}H_{82}NO_6$	 $C_{42}H_{82}NO_6P$ Mw = ~2938.57 g/mol (Average) Exact Mass = ~2937.79 g/mol (Average)	648.07	647.59
LNP11	DLin-DMA 18:2	 $C_{42}H_{82}NO_6$	DSPC $C_{42}H_{82}NO_6P$	616.07	615.80
LNP12	DLin-MC3-DMA (Diosgenin) 18:2	 $C_{42}H_{82}NO_6$	 $C_{42}H_{82}NO_6P$ Mw = 780.18 g/mol Exact Mass = 759.42 g/mol	642.11	641.61
LNP14	ALC-0315 (Diamantyl)	 $C_{42}H_{82}NO_6$	Cholesterol $C_{27}H_{46}O$	766.29	755.72
LNP15	SM-102 (Spikevax)	 $C_{42}H_{82}NO_6$	 $C_{42}H_{82}NO_6P$ Mw = 386.65 g/mol Exact Mass = 359.35 g/mol	710.18	739.66

Figure 4 (Cont.)



LNP ID	Z _{avg} (Size, nm)		SD	
Profile	2-8°C, 0Hz	2-8°C, 4Hz	2-8°C, 0Hz	2-8°C, 4Hz
LNP1	88.58	88.58	0.7593	0.5901
LNP10	77.81	85.54	0.9746	0.0804
LNP11	74.93	88.58	0.1299	0.0231
LNP12	88.01	88.01	0.9608	0.5908
LNP14	112.50	114.53	1.3000	0.2151
LNP15	78.65	78.12	0.1143	1.1441

LNP ID	PDI		SD	
Profile	2-8°C, 0Hz	2-8°C, 4Hz	2-8°C, 0Hz	2-8°C, 4Hz
LNP1	0.0027	0.0141	0.0316	0.0868
LNP10	0.0108	0.0141	0.0143	0.0144
LNP11	0.0005	0.0131	0.0139	0.0181
LNP12	0.0153	0.0123	0.0177	0.5908
LNP14	0.1104	0.0682	0.0148	0.0251
LNP15	0.0042	0.0023	0.0019	0.0048

PDI: Polydispersity index

LNP ID	Charge (ZP, mV)		SD	
Profile	2-8°C, 0Hz	2-8°C, 4Hz	2-8°C, 0Hz	2-8°C, 4Hz
LNP1	21.24	24.26	0.9016	0.7051
LNP10	-5.29	-5.48	2.4709	0.6172
LNP11	10.00	10.31	0.7389	1.0631
LNP12	1.78	2.20	1.0200	0.0001
LNP14	18.01	18.25	1.8230	0.2780
LNP15	-0.89	-4.63	0.9069	0.0097

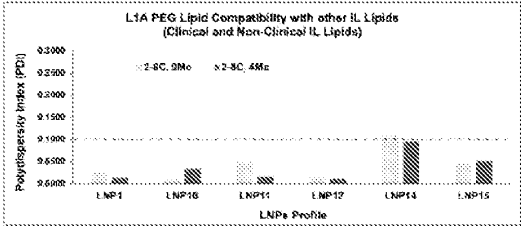
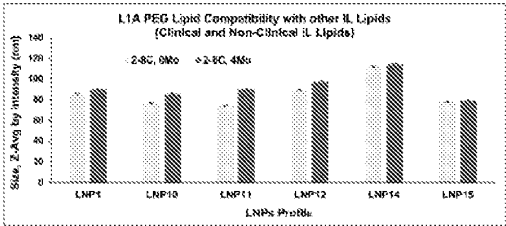
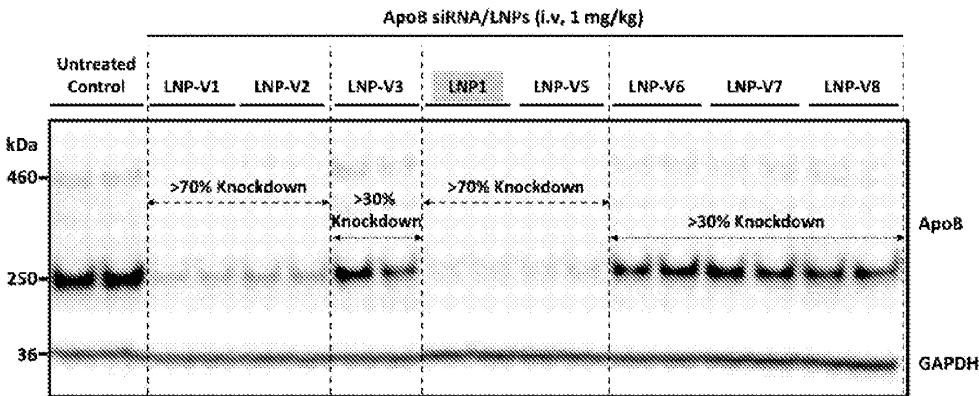
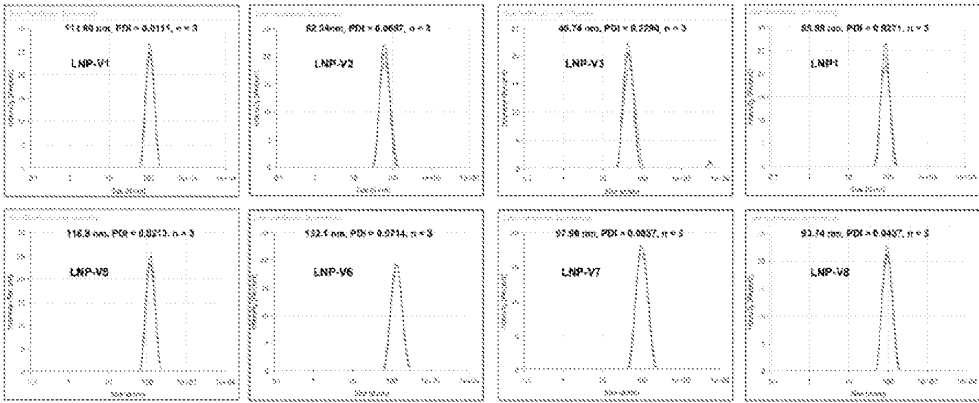


Figure 5
Flexibility with Four Lipid Component Molar Ratios of LNP-1 (Exemplary)



Varied Molar Ratios of Four Lipid Components of LNP-1 (Exemplary)				
LNP ID	DODMA	DSPC	Cholesterol	PEGylated L1A
LNP-V1	59.25	10	30	0.75
LNP-V2	50	10	38	2
LNP-V3	50	10	35	5
LNP1	50	10	39.25	0.75
LNP-V5	55	5	39.25	0.75
LNP-V6	10	50	39.25	0.75
LNP-V7	5	55	39.25	0.75
LNP-V8	30	10	59.25	0.75

Figure 5 (Cont.)



LNP ID	Z _{avg} (Size, nm)	SD
Profile	Freshly-formulated	
LNP-V1	111.6	0.466
LNP-V2	82.54	0.8446
LNP-V3	66.74	2.277
LNP1	81.98	3.7660
LNP-V5	116.3	1.798
LNP-V6	132.1	3.5065
LNP-V7	97.98	3.3521
LNP-V8	93.74	3.2183

LNP ID	PDI	SD
Profile	Freshly-formulated	
LNP-V1	0.0111	0.0055
LNP-V2	0.0687	0.0300
LNP-V3	0.2289	0.0439
LNP1	0.0271	0.0216
LNP-V5	0.0212	0.0126
LNP-V6	0.0714	0.0325
LNP-V7	0.0837	0.0305
LNP-V8	0.0437	0.0153

PDI: Polydispersity Index

LNP ID	Charge (ZP, mV)	SD
Profile	Freshly-formulated	
LNP-V1	35.77	3.70
LNP-V2	26.52	6.79
LNP-V3	17.13	7.71
LNP1	21.24	6.50
LNP-V5	3.60	6.79
LNP-V6	39.92	5.61
LNP-V7	23.37	4.71
LNP-V8	8.49	0.22

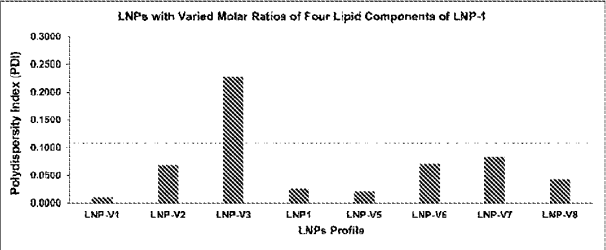
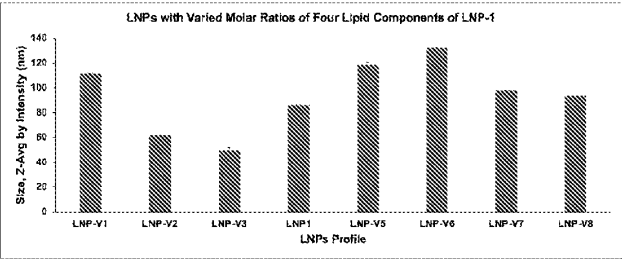


Figure 6

Flexibility with Microfluid Buffer of LNP-1 (Exemplary)

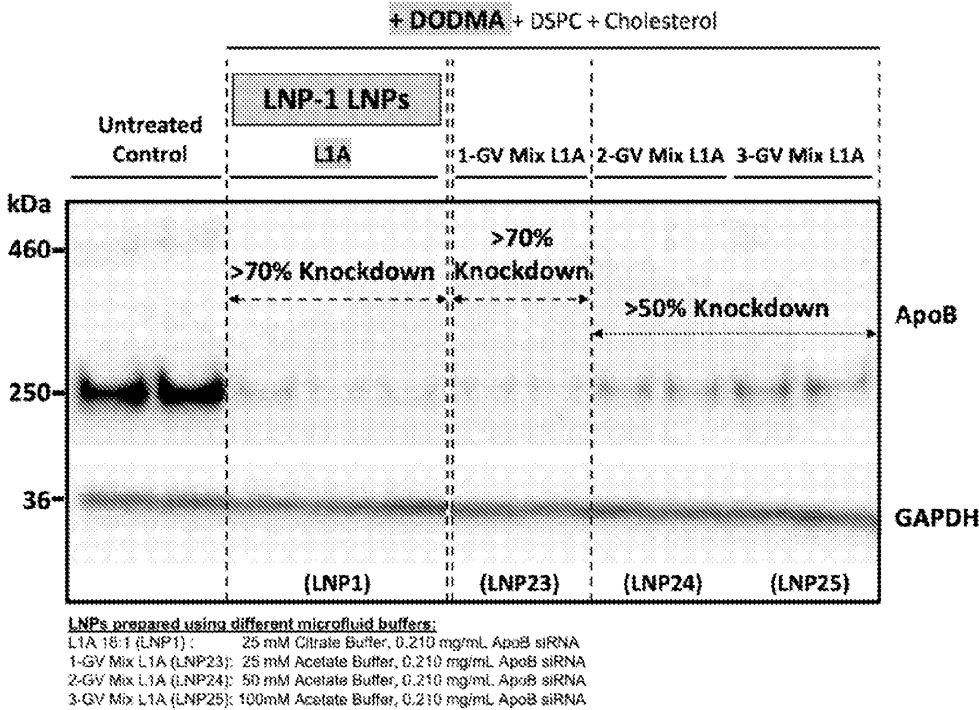
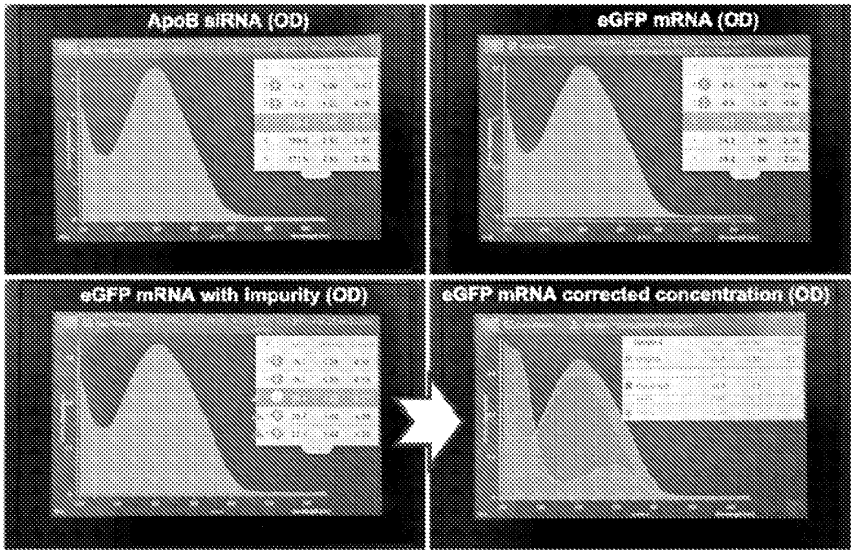
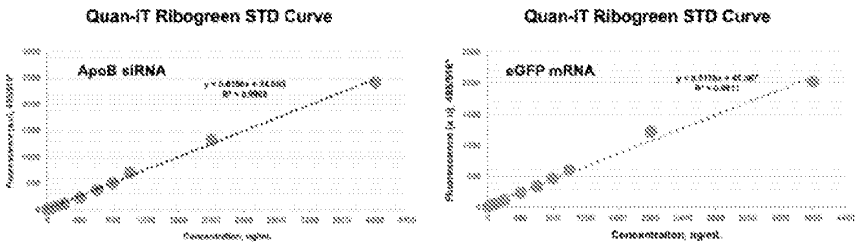


Figure 7

RNA Payload Assay for LNP-1 LNPs Formulations
Fluorescent Quant-iT RNA RiboGreen Quantification Method (Exemplary)



OD measurements by NanoDrop One from Thermo Fisher Scientific (Exemplary)



Fluorescent Quant-iT RNA RiboGreen Quantification Method STD Curve (Exemplary)

Figure 7 (Cont.)

1. Quantify a stock solution (e.g. 20-1000ng/uL) by OD (Optical Density) at A_{260} , A_{280} , A_{260}/A_{280} and A_{260}/A_{230} , see NanoDrop One exemplary spectra data images. Note: Apply a corrected RNA concentration if any impurity is present in the RNA samples, specifically for mRNAs, as shown on spectra data image.
2. Prepare in a fluorescent microplate assay (96-well plate) a series of diluted concentrations from the above RNA stock solution for building a Standard Curve (e.g. 0-2000+ng/mL). Add as well LNPs-RNAs stock solution as control, disassembled LNP-RNA samples from stock solution and/or other control samples in the black clear bottom microplate. Note: Disassembled LNP-RNA samples are incubated at 5% (v/v) TX-100 in TE Buffer (1x) at 50°C for 2h, then diluted at a final 0.1% (v/v) TX-100 concentration in TE Buffer (1x).
3. Ultrasensitive Fluorescent Quan-iT RNA Ribogreen Quantification Method is performed to all RNAs samples e.g. Standard Curve (STD as shown on plots images) vs LNPs-RNAs control vs disassembled LNP-RNA samples vs other control samples according to vendor protocol. Note: Take into consideration any effect of common contaminants for this ultrasensitive fluorescent assay, see link:
<https://assets.thermofisher.com/TFS-Assets/LSG/manuals/mp11490.pdf>

Figure 8

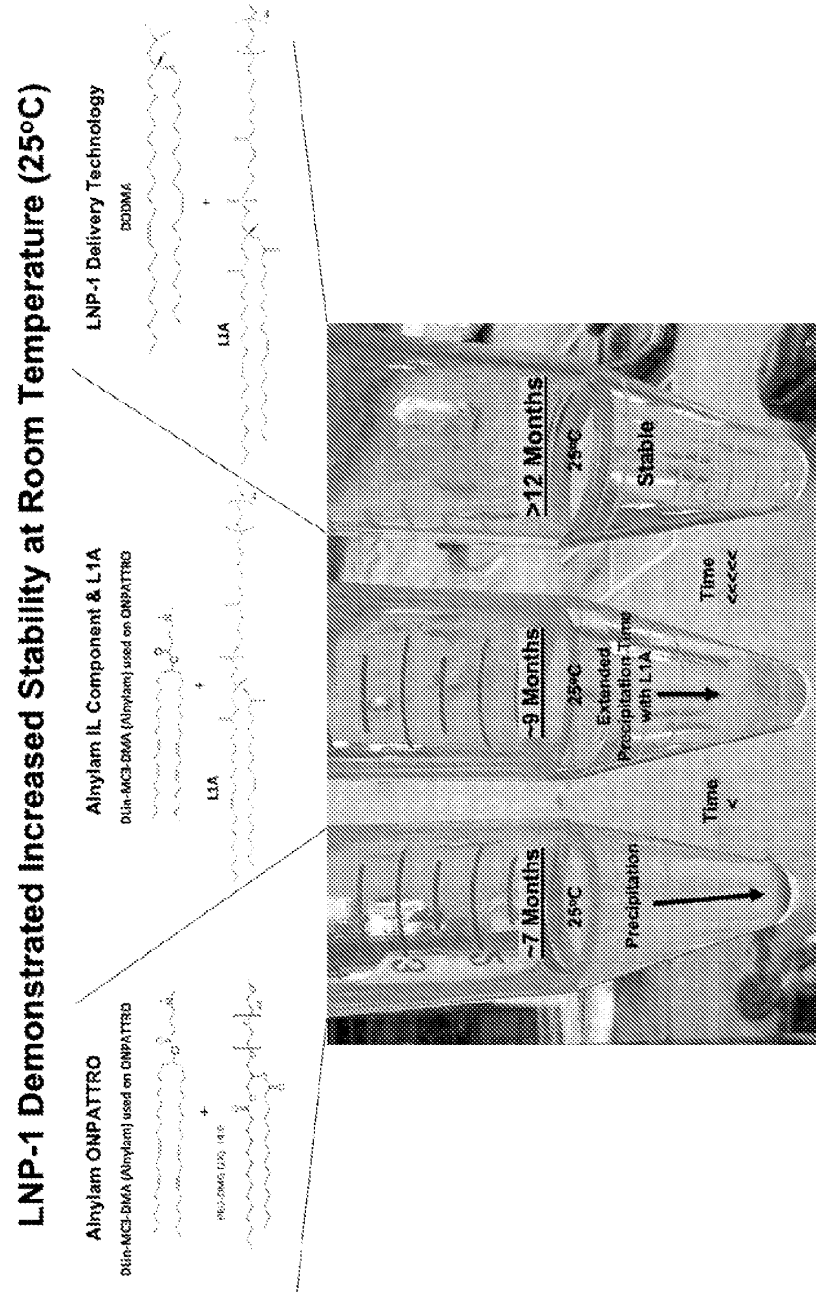


Figure 9

Structural & Functional Stable L1A PEG-lipid is Compatible with Other Ionizable Lipids Having Smaller Sizes

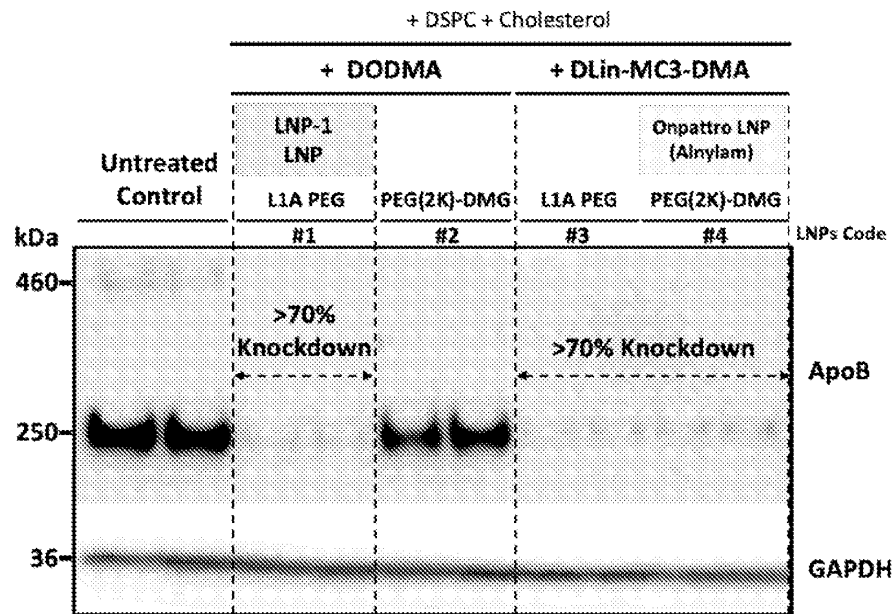


Figure 9 (Cont.)

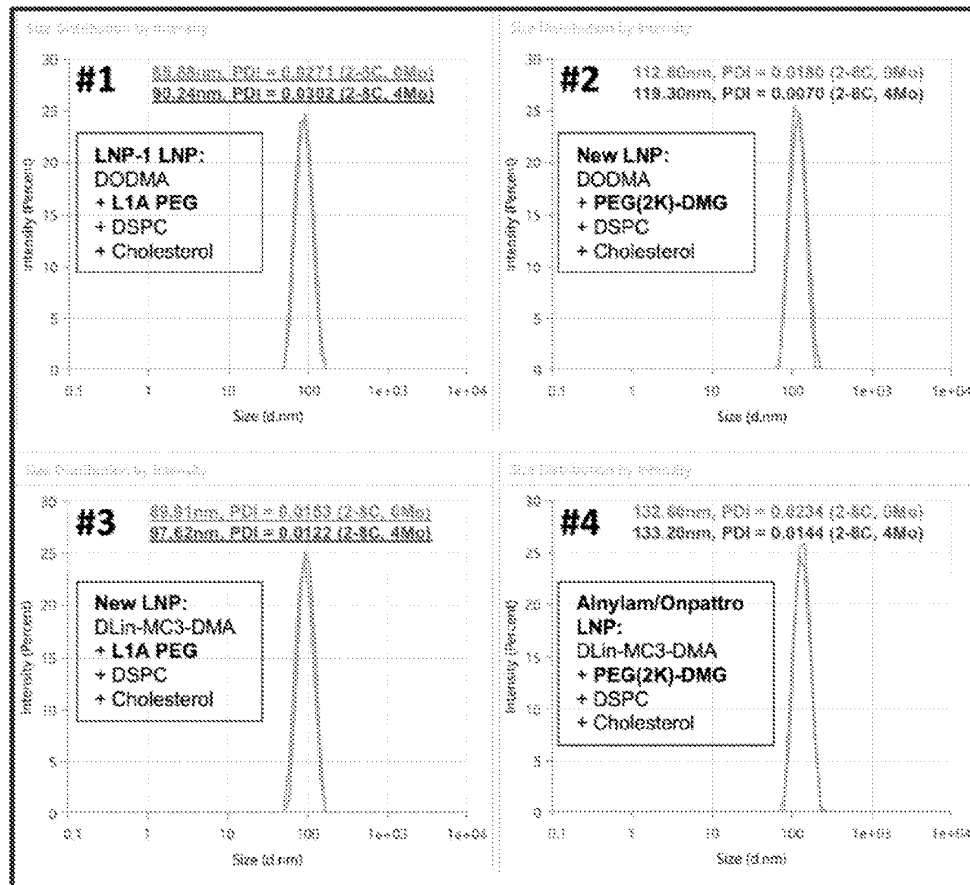


Figure 9 (Cont.)

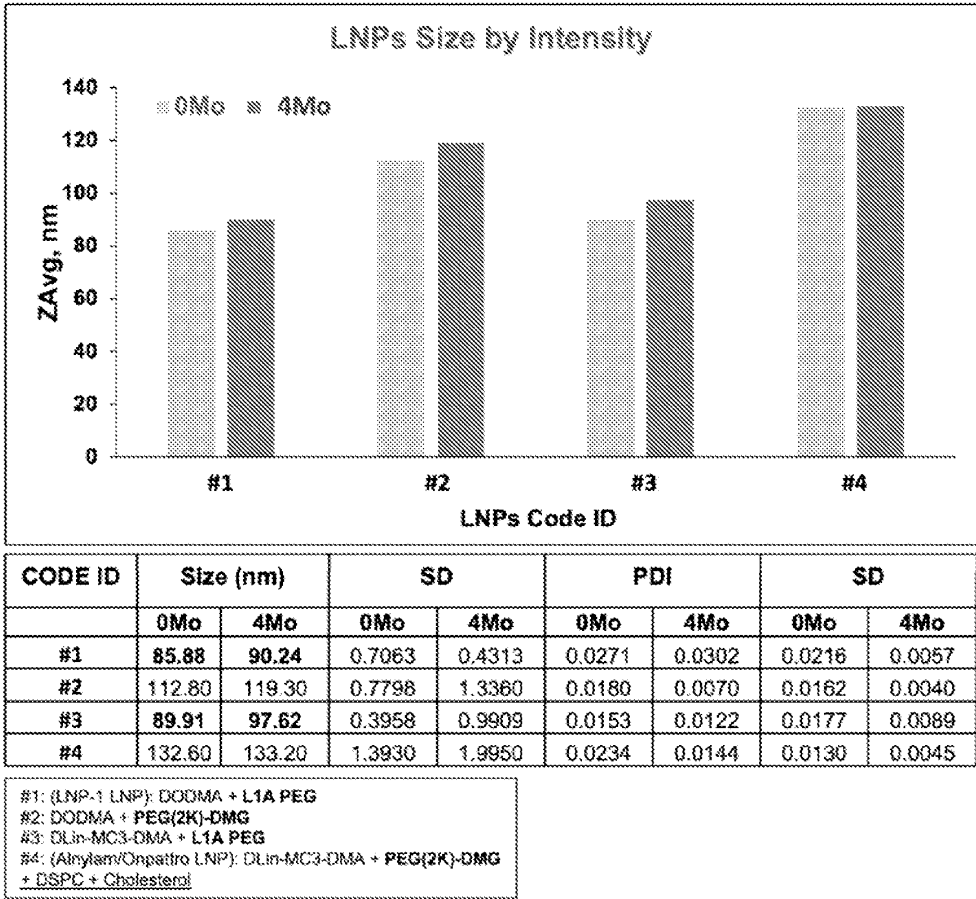


Figure 10

Long-term Structural Lipids Stability and Quantification
– Stable Lipid Composition of Aged eGFP mRNA/LNP-1

Sample	Lipid Name	Calculated Concentration (mg/ml)	LNP Total Mass (mg/ml)	MW	mmol in 1ml	Total Count (mmol)	Assay mmol%	Assay m/m%
eGFP mRNA/ LNP-1 (Freshly-formulated)	DOHMA	3.254	6.448	620.10	0.0005830	6.001256	46.41	50.46
	DSPC	1.024		790.15	0.0001480		11.48	13.88
	Cholesterol	1.803		385.65	0.000191		41.33	27.94
	L1A	0.245		2841.05	0.000096		0.76	3.80
Sample	Lipid Name	Calculated Concentration (mg/ml)	LNP Total Mass (mg/ml)	MW	mmol in 1ml	Total Count (mmol)	Assay mmol%	Assay m/m%
eGFP mRNA/LNP-1 (2-8°C, 9 Months Storage)	DOHMA	2.735	5.547	620.10	0.0004874	6.001135	42.94	49.04
	DSPC	0.687		790.15	0.0000965		8.51	12.39
	Cholesterol	1.891		385.65	0.0005447		47.98	34.09
	L1A	0.197		2841.00	0.0000615		0.54	2.84

Figure 11
Long-term Physicochemical Stability of Aged LNP-1 with Different RNA Payloads

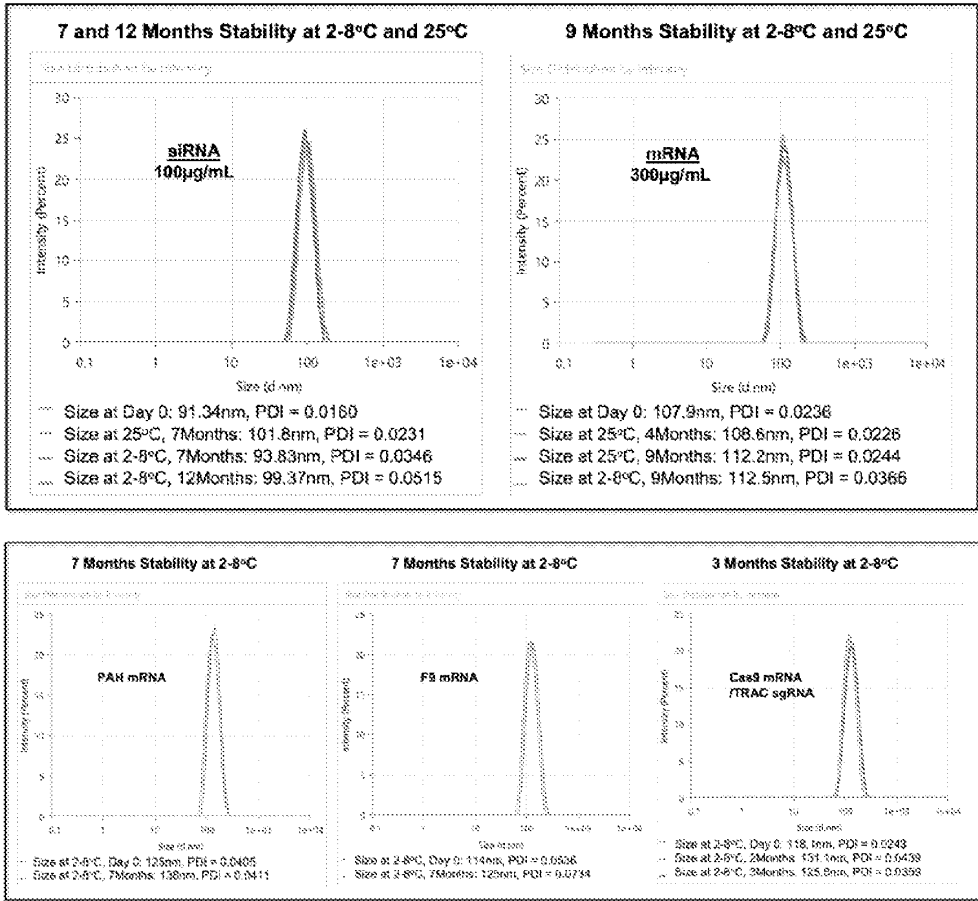
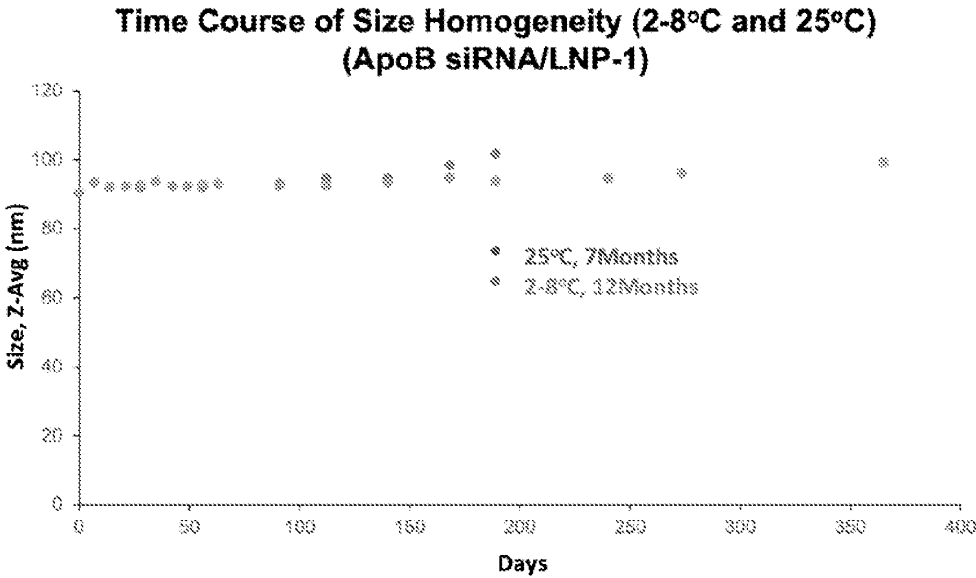


Figure 12



**Physical Characterization of Critical Quality Attributes Summary (25°C)
(ApoB siRNA/LNP-1)**

Days	Size Zavg ± SD (nm)	PDI ± SD	Average Charge Zeta Potential ± SD (mV)	Average Conductivity
0	91.34 ± 0.2126	0.0160 ± 0.0012	-26.9097 ± 7.1642	0.3802 ± 0.1530
7	90.73 ± 0.8719	0.0363 ± 0.0132		
28	90.65 ± 0.5808	0.0121 ± 0.0114		
56	93.45 ± 0.1841	0.0313 ± 0.0140		
84	96.65 ± 1.218	0.0589 ± 0.0107		
112	98.65 ± 0.2878	0.0716 ± 0.0183		
140	102.9 ± 0.9965	0.0793 ± 0.0440		
168	101.5 ± 0.6061	0.0693 ± 0.0096		
189	103.2 ± 0.6065	0.0836 ± 0.0111		

Figure 13

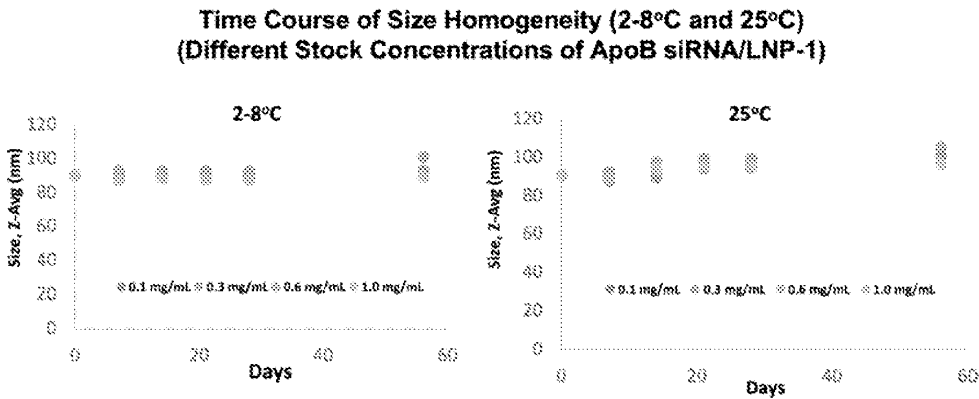
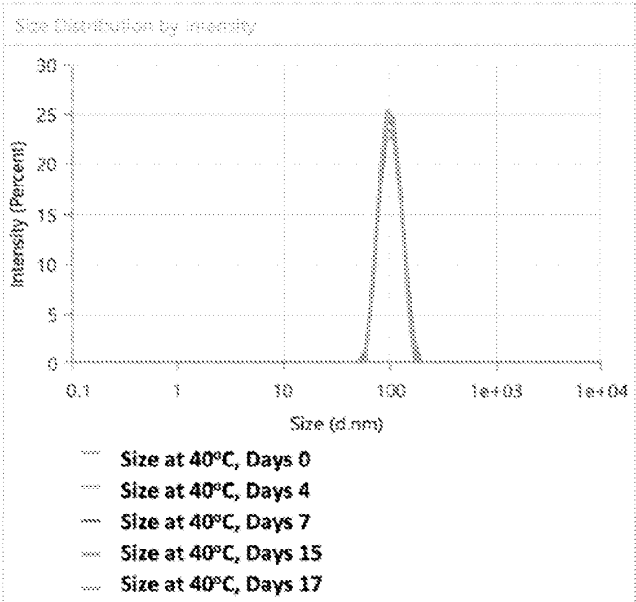
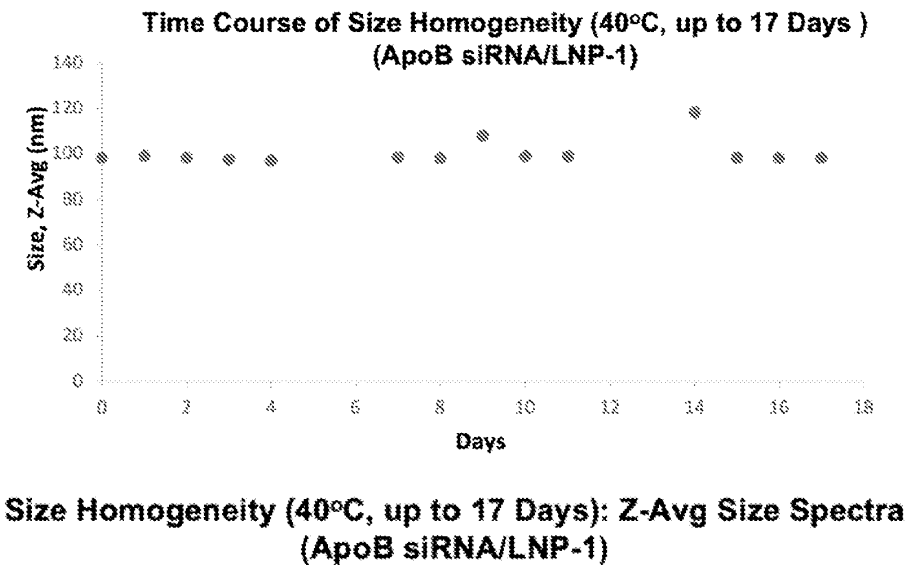


Figure 14

Long-term Stability of ApoB siRNA/LNP-1 at 40°C



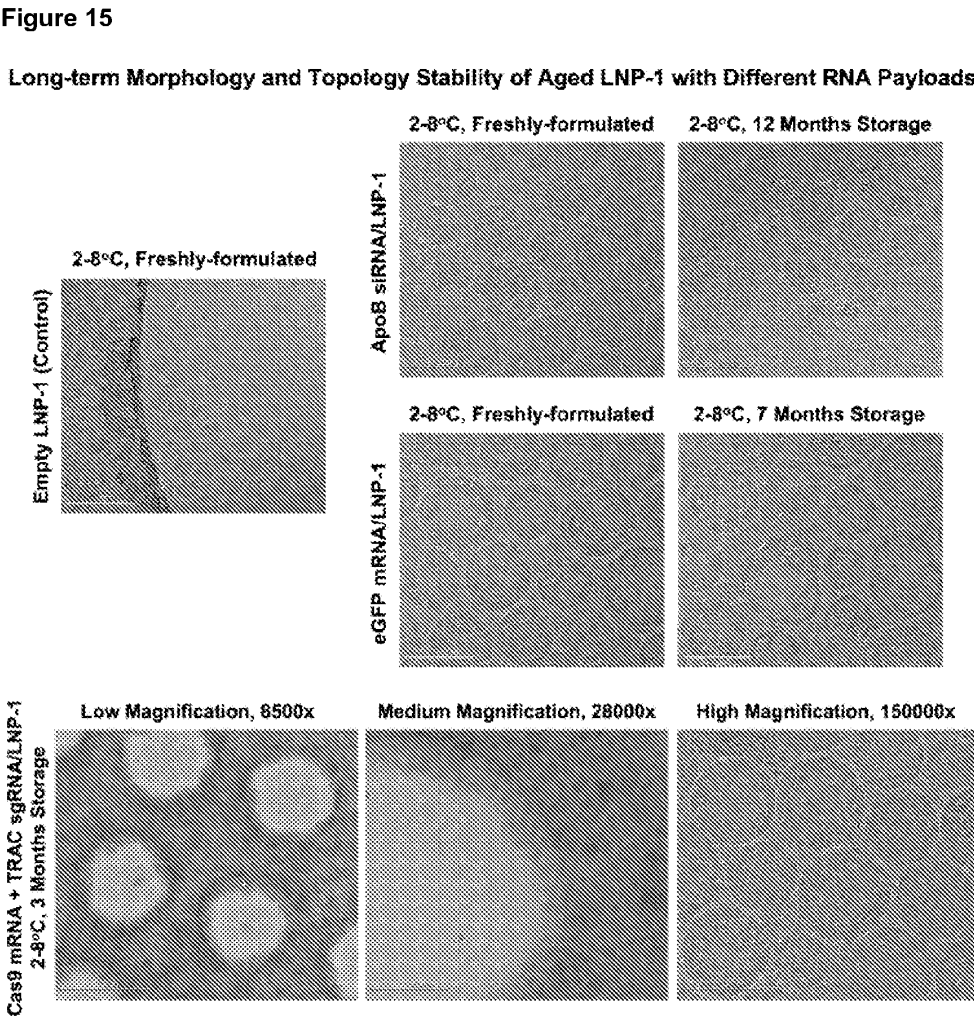


Figure 16

LNP-1 Platform Characterization Summary:
Payloads Versatility and Long-term Stability

Payload Type	Payload Size (nt)	Encapsulation Efficiency	RNA/LNP-1 Size (nm)	Long-term Stability (2-8°C)		
ApoB siRNA	21 (ds)	>94%	90 ± 10	12Months	PDI	0.0515
					Zeta Potential (mV)	35.02 ± 0.6746
eGFP mRNA	980 (ss)	>87%	117 ± 10	9Months	PDI	0.0366
					Zeta Potential (mV)	32.66 ± 1.234
PAH mRNA	1619 (ss)	>69%	129 ± 10	7Months	PDI	0.0411
					Zeta Potential (mV)	32.89 ± 1.743
P9 mRNA	1646 (ss)	>65%	125 ± 10	7Months	PDI	0.0734
					Zeta Potential (mV)	34.75 ± 1.065
Cas9 mRNA/sgRNA	4471/100 (ss/ss)	>80%/>90%	125 ± 10	3Months	PDI	0.0359
					Zeta Potential (mV)	36.32 ± 0.5806

ds: Double-Stranded
ss: Single-Stranded

Figure 17

Long-term Function Stability – Stable CAR-T Generation Potency of Aged eGFP mRNA/LNP-1

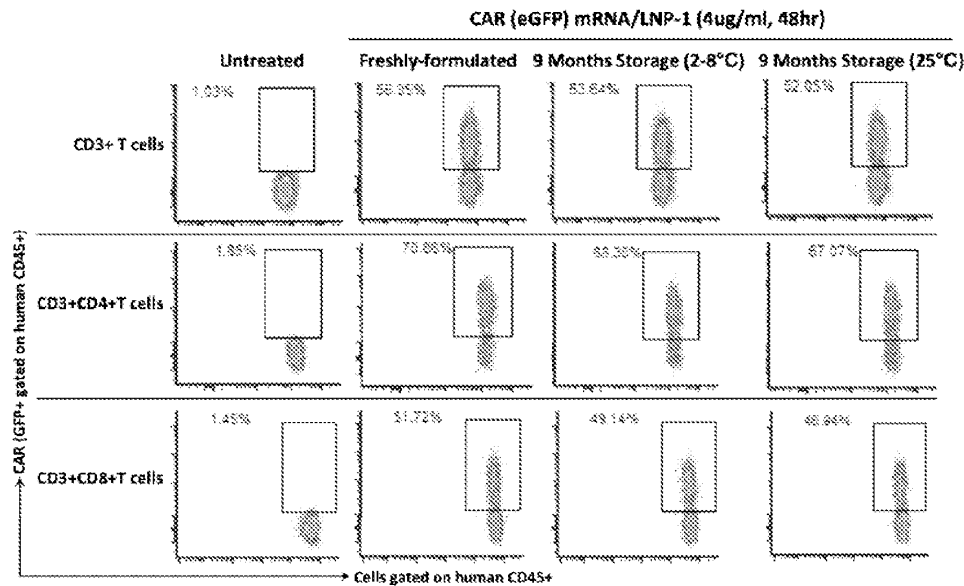


Figure 18
Long-term Function Stability – Stable Gene Delivery Potency of Aged eGFP mRNA/LNP-1

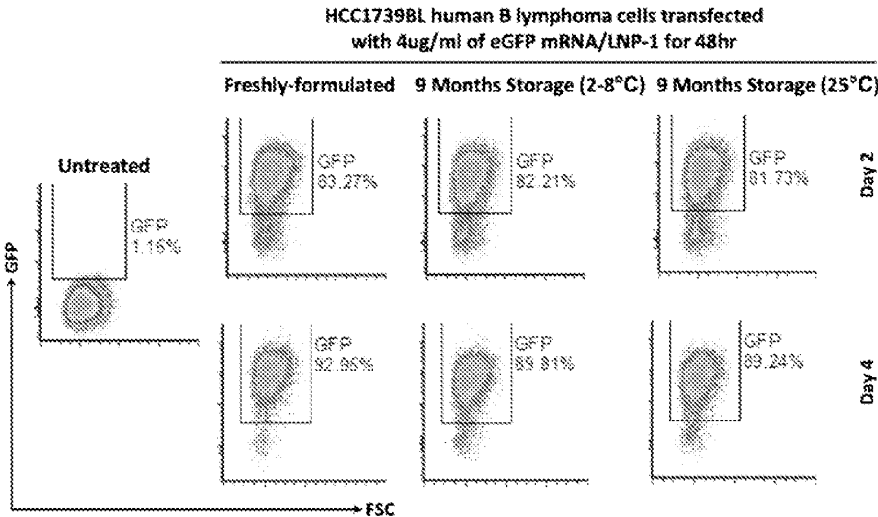


Figure 19

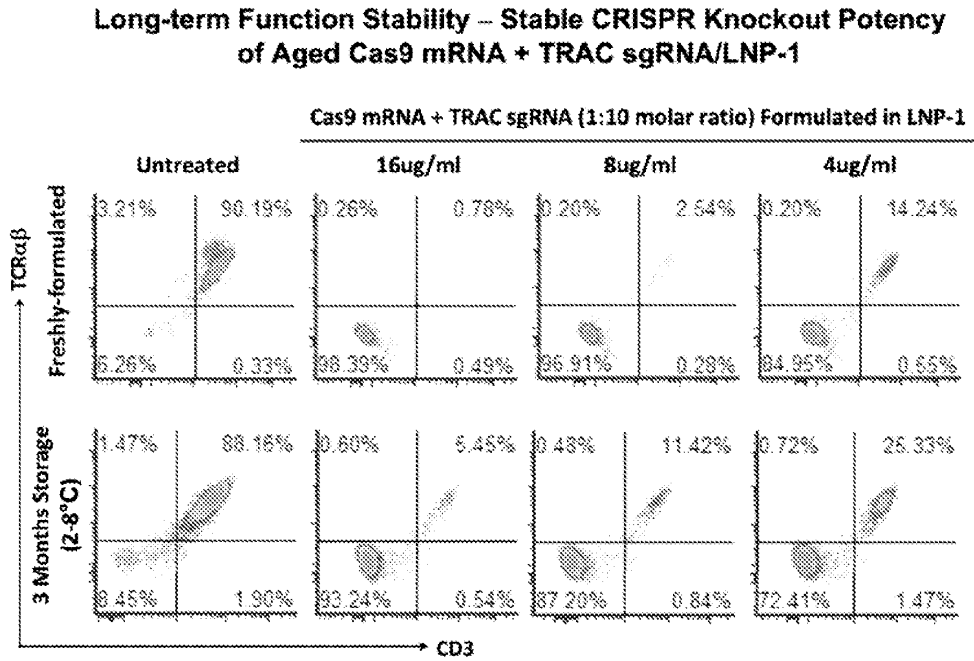


Figure 20

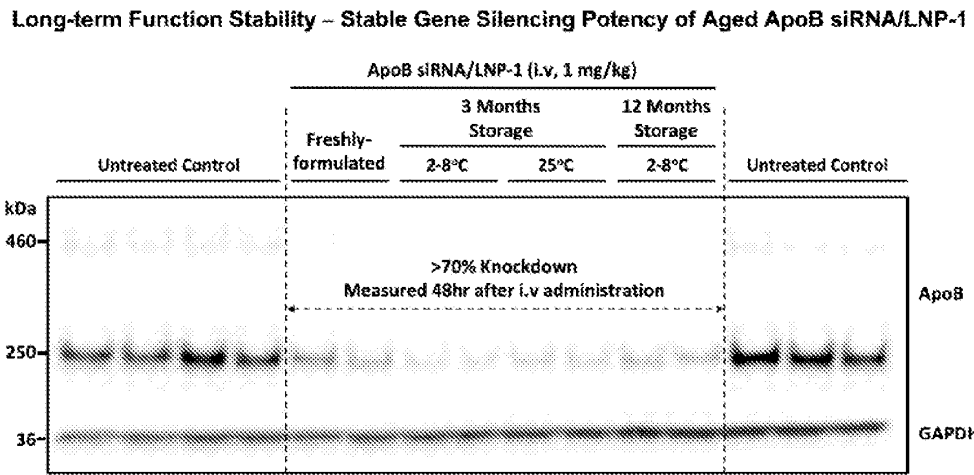
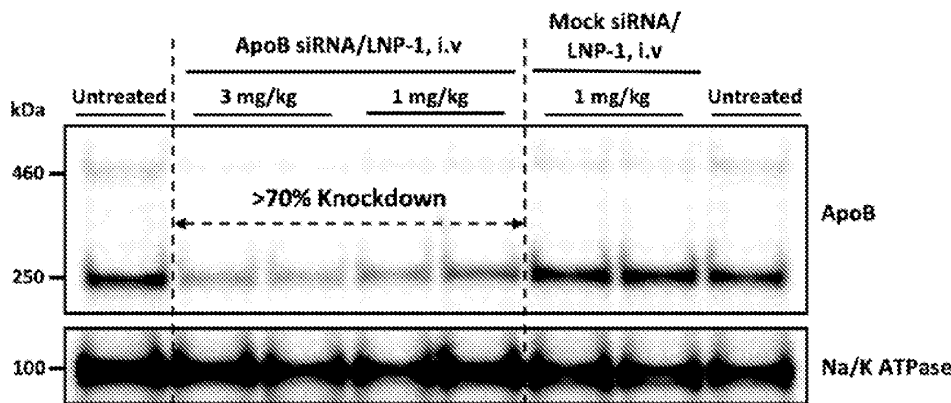


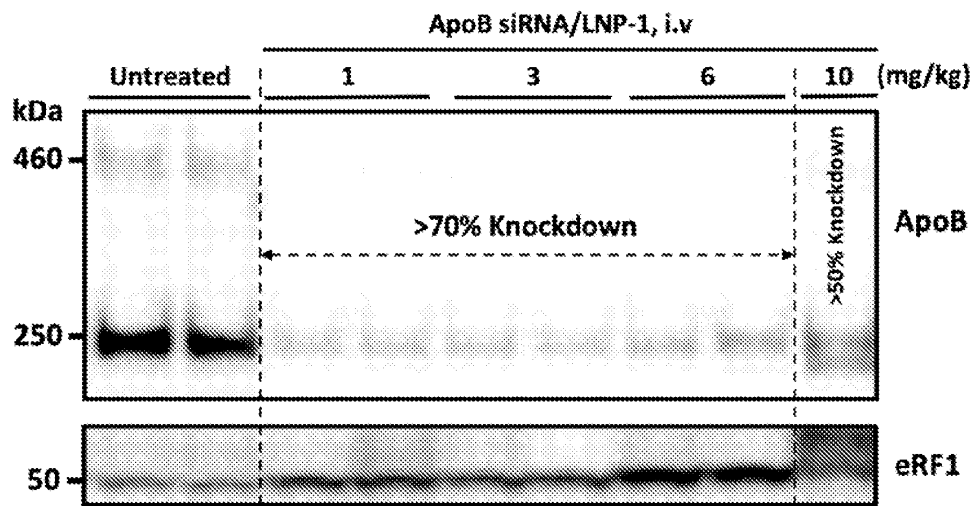
Figure 21

LNP-1's Versatile Therapeutic Applications – Gene Silencing

In Vivo Knockdown Potency of ApoB siRNA/LNP-1 in Mouse Livers



ApoB siRNA/LNP-1 In Vivo Potency at Different Dosages



Note: No abnormality in biochemistry profiles was detected in 1-3 mg/kg treated mice.

Figure 22

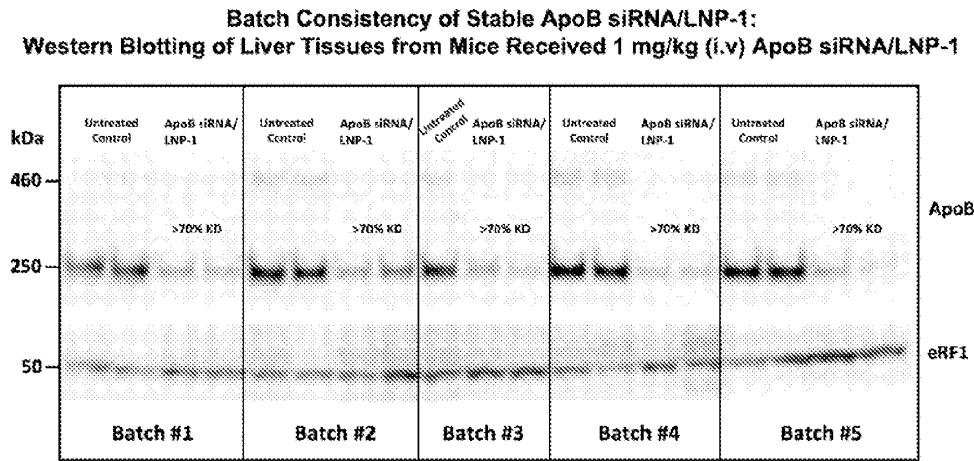


Figure 23

LNP-1's Versatile Therapeutic Applications – mRNA Therapy
In Vivo Gene Delivery Potency of LNP-1 (Exemplary)

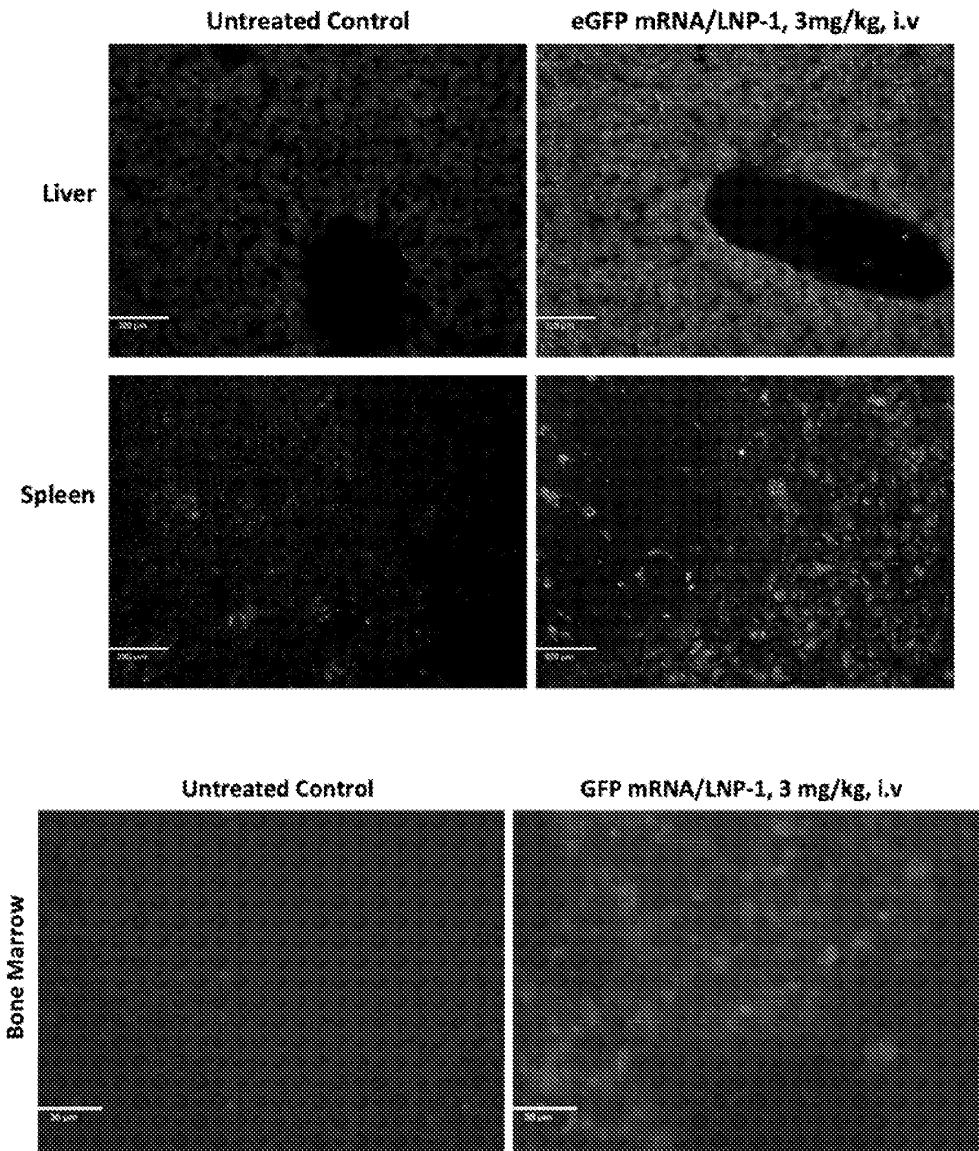


Figure 24

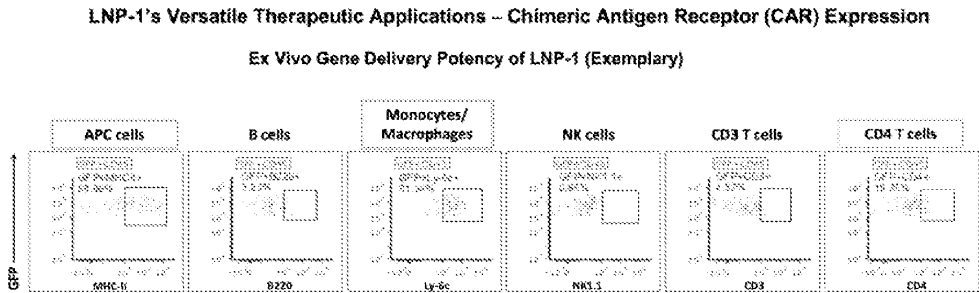


Figure 25

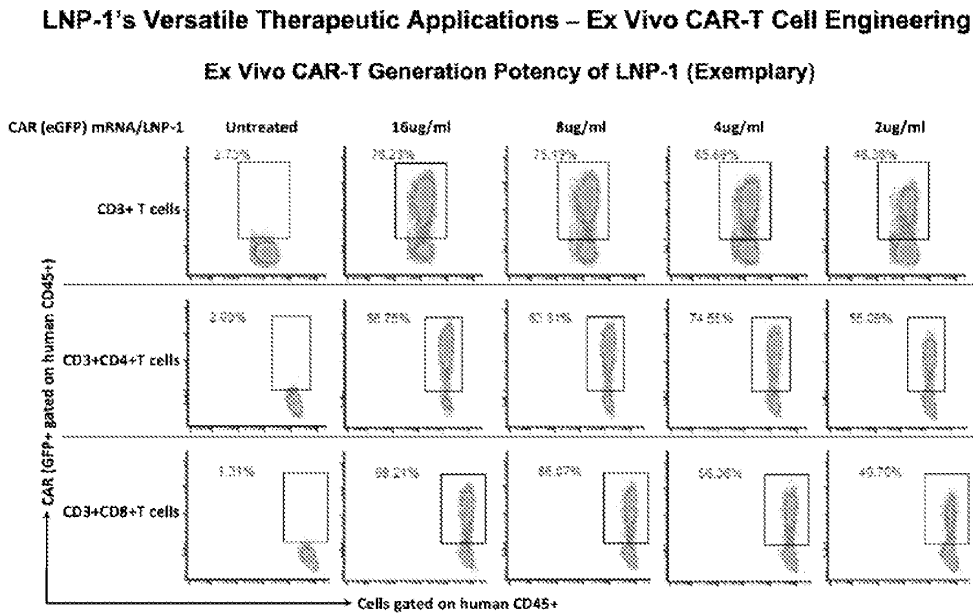


Figure 26

LNP-1's Versatile Therapeutic Applications – In Vivo CAR-T Cell Engineering

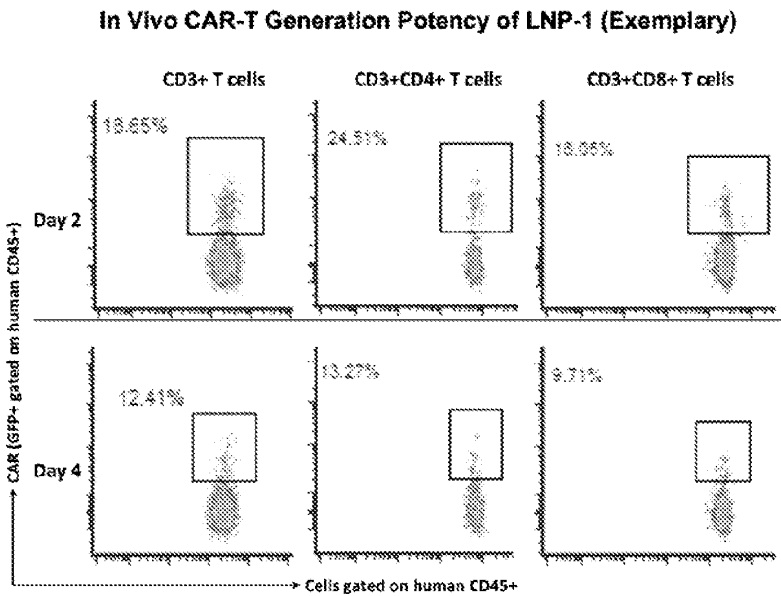


Figure 27

LNP-1's Versatile Therapeutic Applications – Stem Cell Engineering

Gene Delivery Potency of LNP-1 to Hematopoietic Stem Cells (Exemplary)

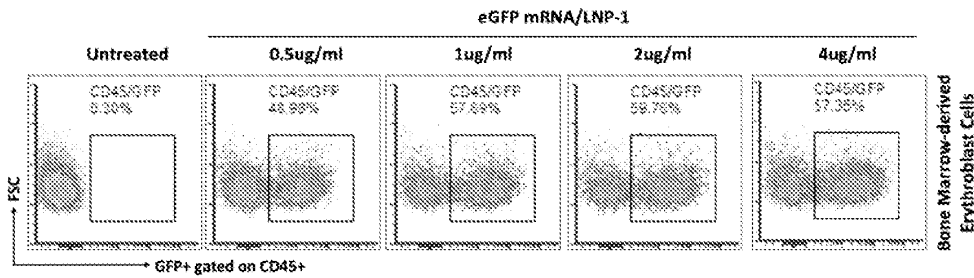


Figure 28

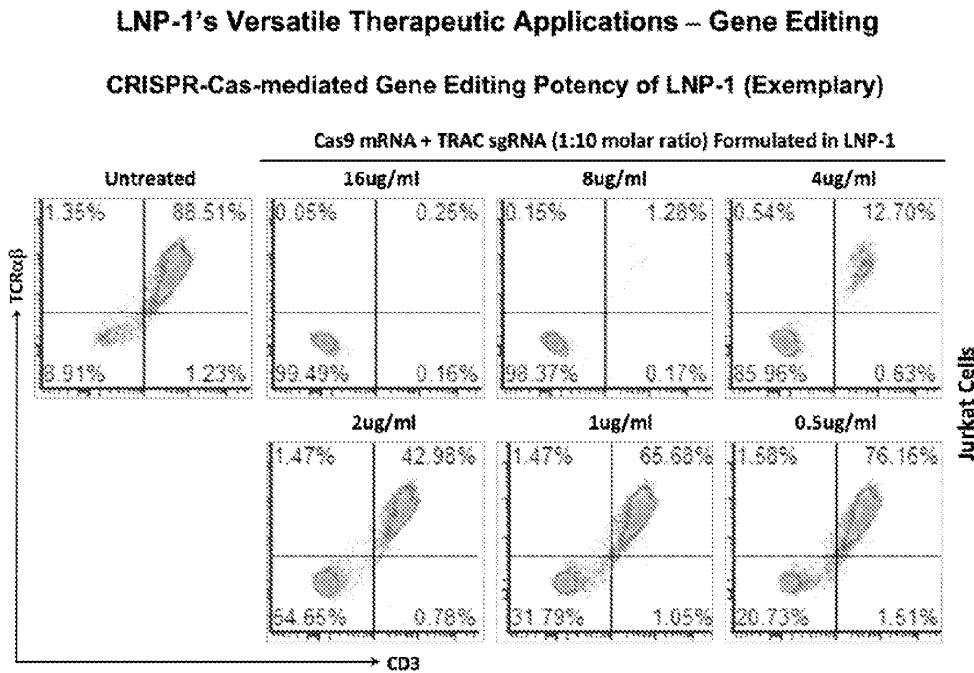


Figure 29

LNP-1's Versatile Therapeutic Applications – Gene Therapy for Malignant Cells
Gene Delivery Potency of LNP-1 to Human Leukemia Cells (Exemplary)

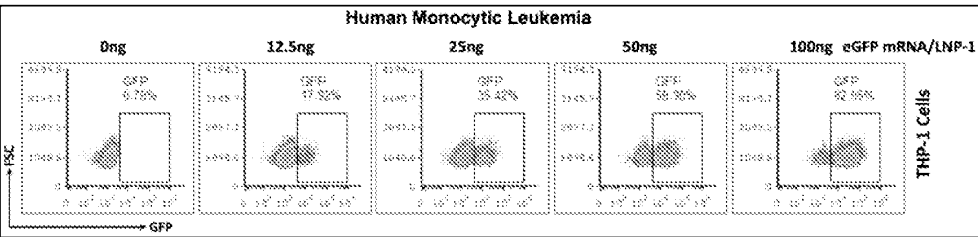
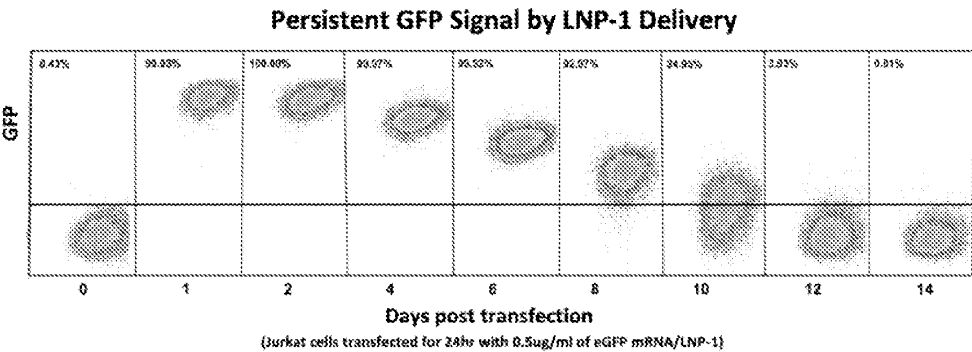
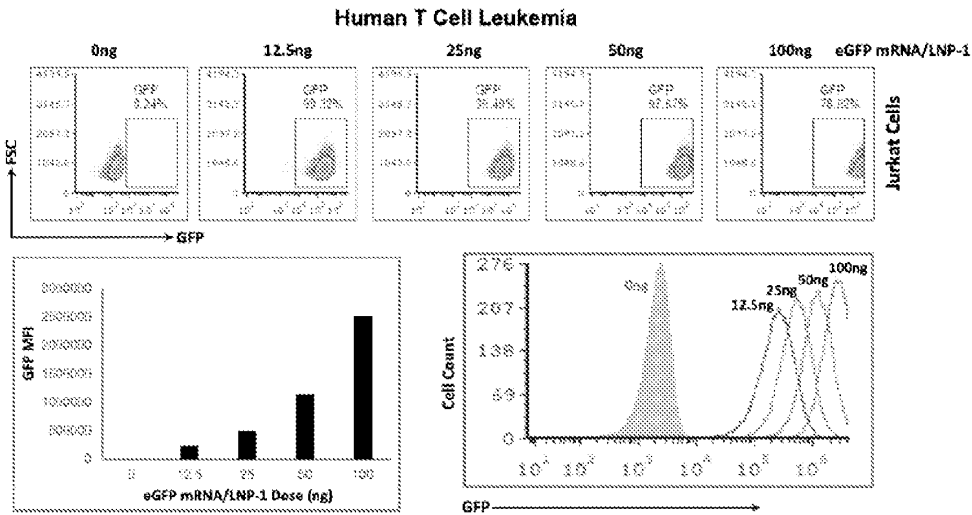


Figure 30

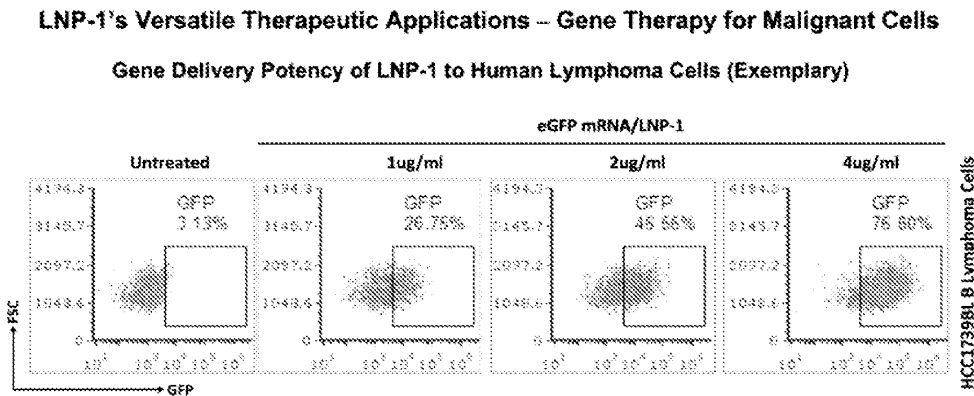


Figure 31

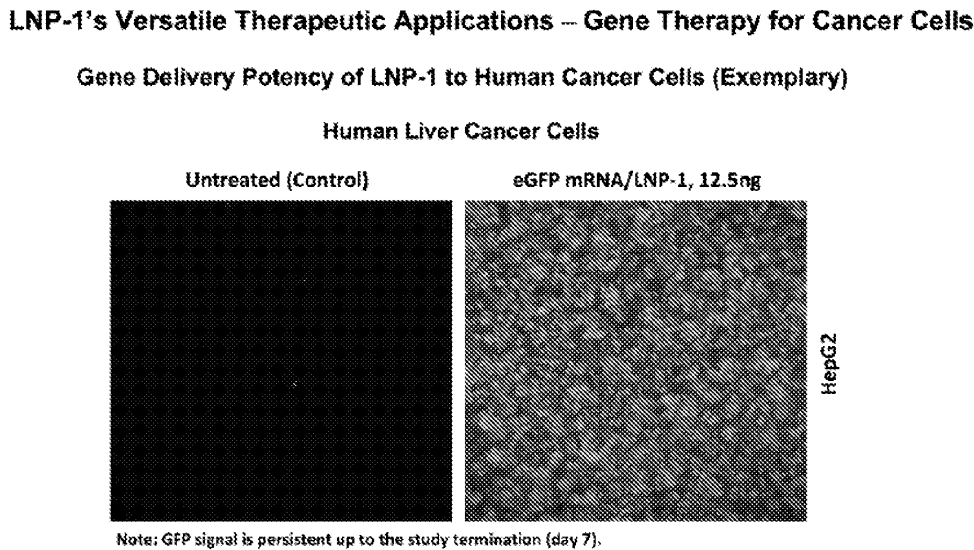


Figure 32

LNP-1's Versatile Therapeutic Applications – Vaccines
LNP-1's Potency as COVID-19 mRNA Vaccines (Exemplary)

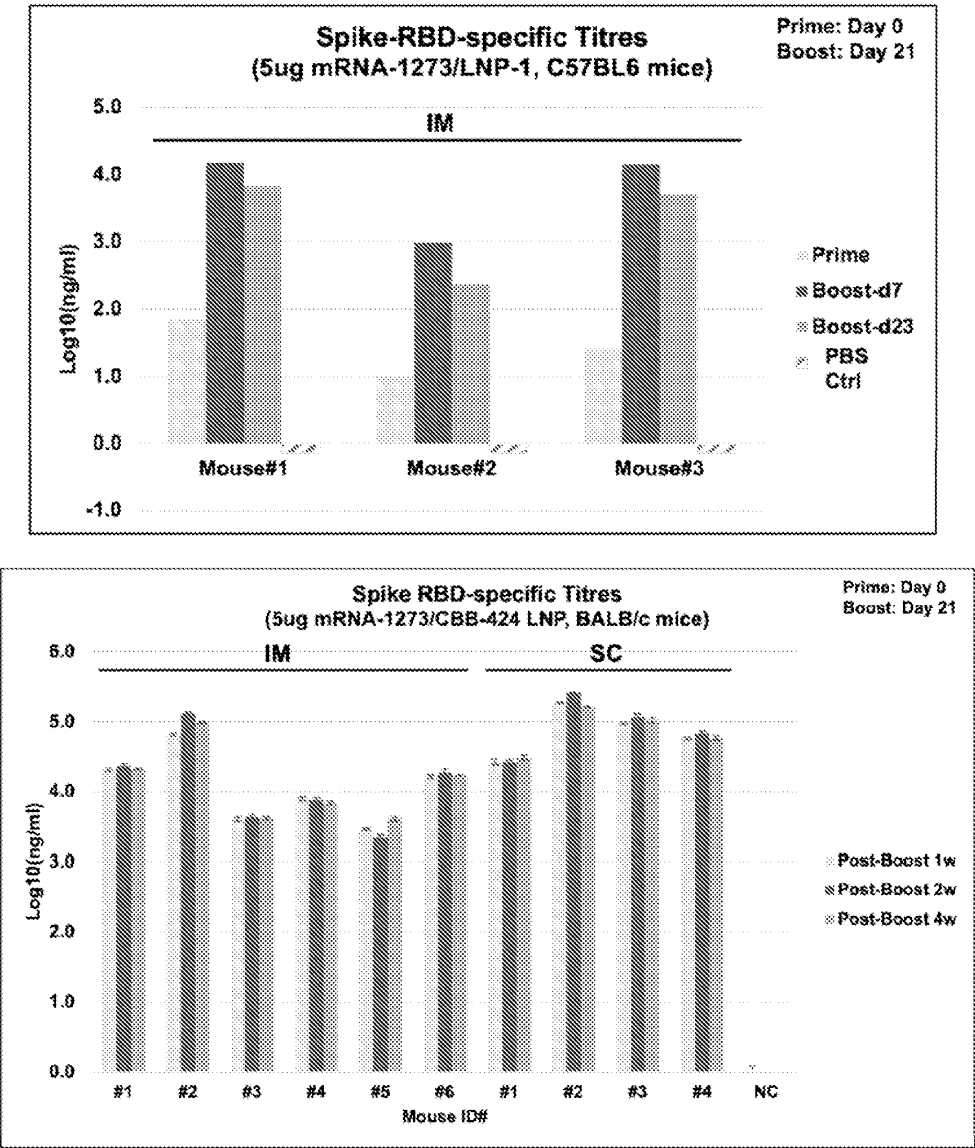


Figure 33

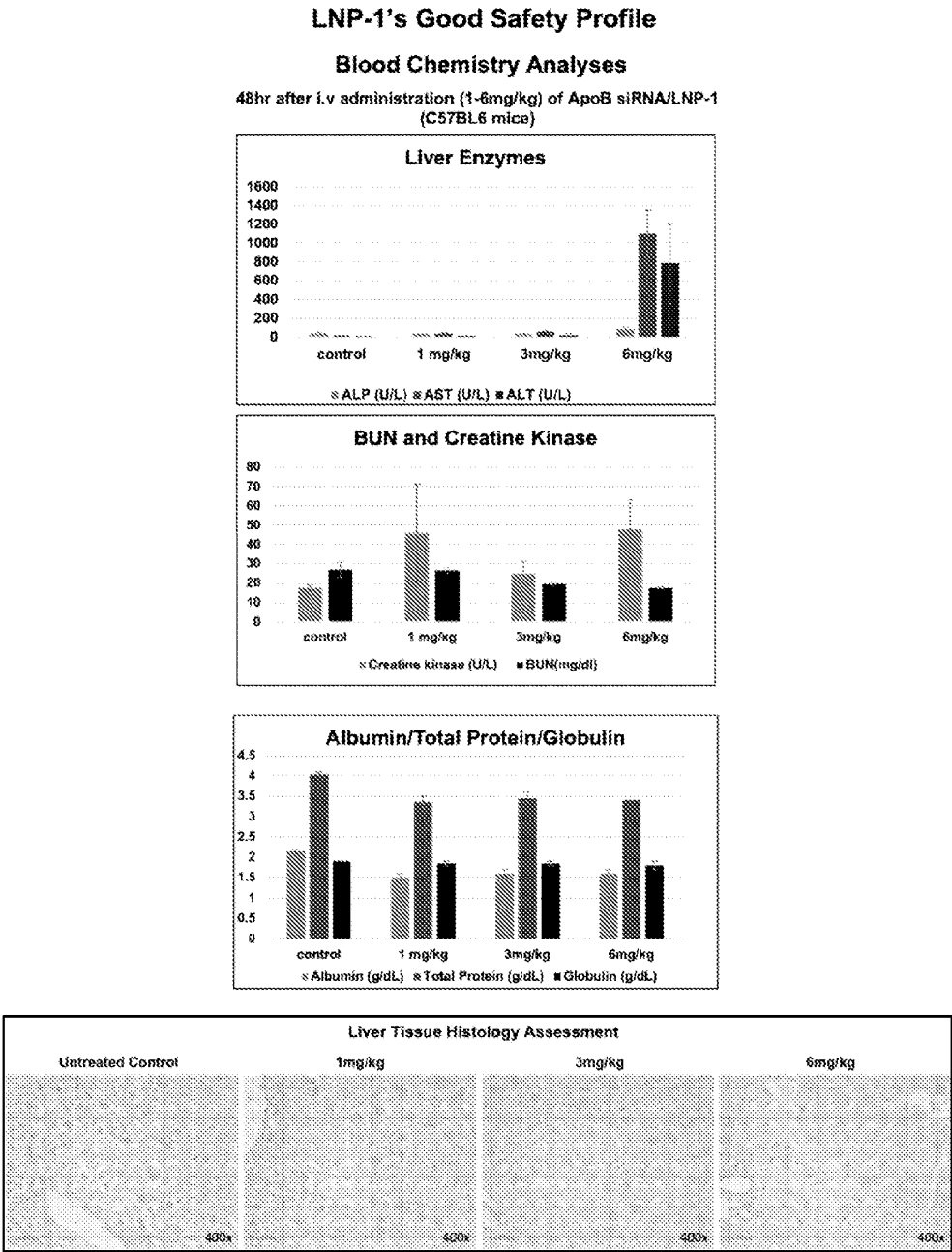


Figure 34

