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Supplemental Information

Lyophilization provides long-term stability for a lipid nanoparticle-formulated, nucleoside-modified mRNA vaccine

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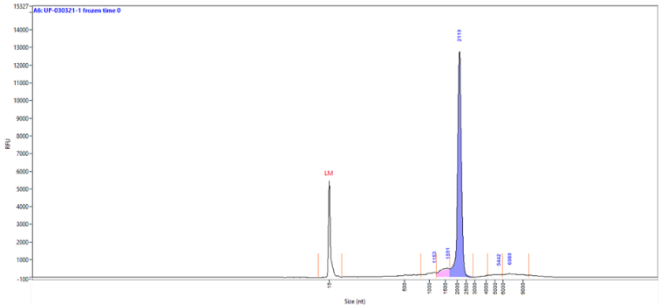
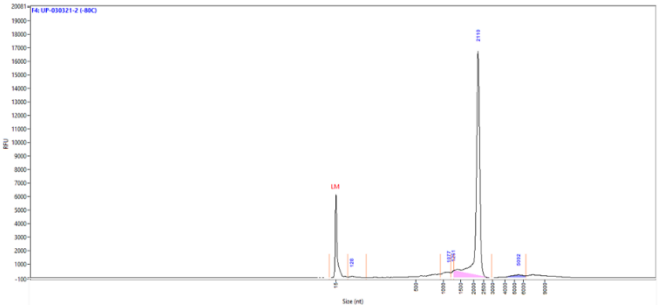
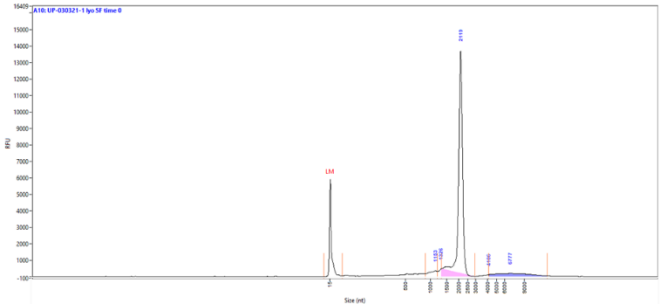
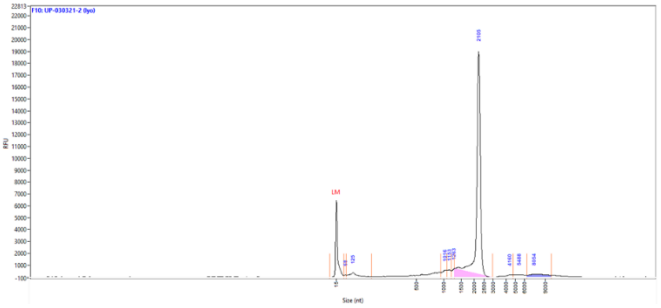
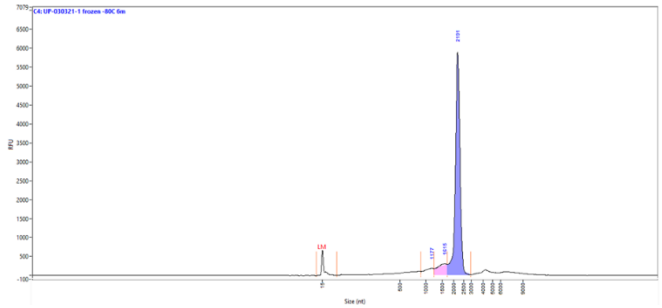
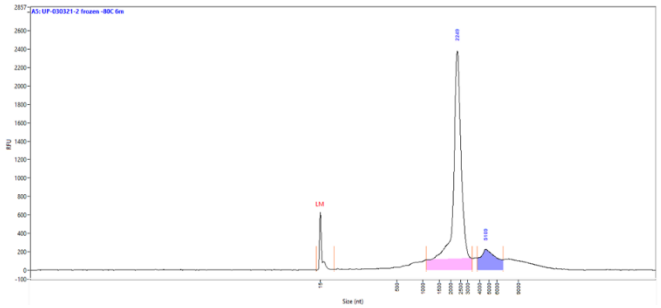
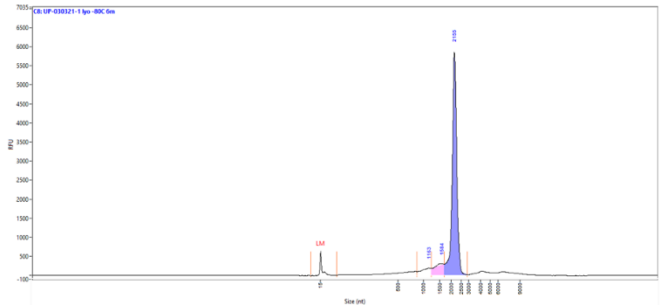
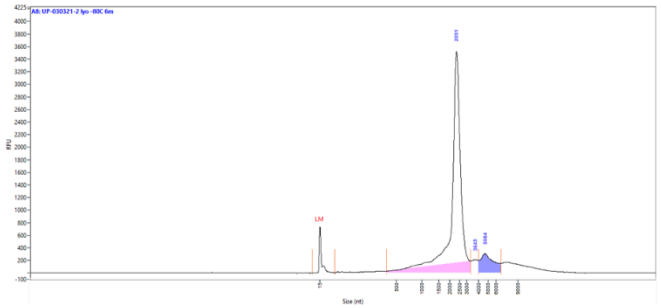
Supplemental Data

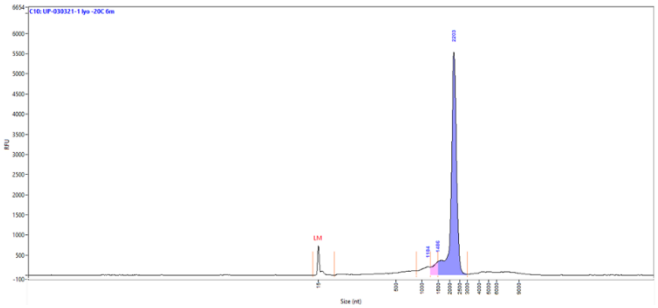
Figure S1. Related to Figure 1. Representative images of lyophilized vials containing Luc mRNA-LNPs. Luc mRNA-LNPs were stored at -80ºC, -20ºC, 4ºC, room temperature (RT, 25ºC) or 42ºC for 0, 4 or 24 weeks. All lyophilized mRNA-LNPs formed a uniform, dense, white cake.

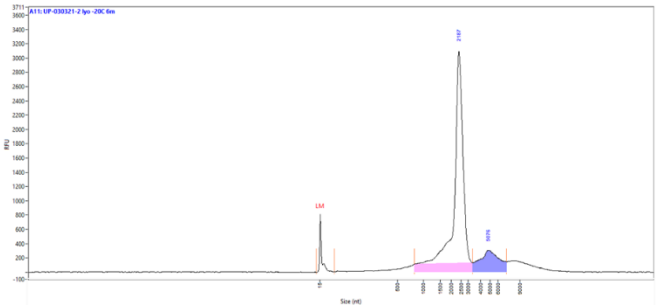
Figure S2. Related to Figure 1. Representative images of reconstituted lyophilized Luc mRNA-LNPs. Luc mRNA-LNPs were reconstituted with nuclease-free water after storage at -80ºC, -20ºC, 4ºC, room temperature (RT, 25ºC) or 42ºC for 0, 4 or 24 weeks. All reconstituted mRNA-LNPs acquired a clear, opalescent appearance with no visible solids.

A

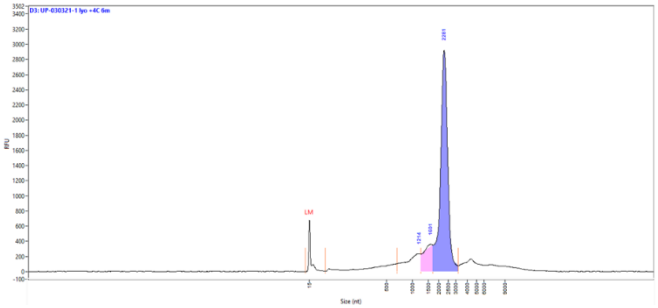
	Luc mRNA-LNP	PR8 HA mRNA-LNP
Frozen (-80ºC) W0		
Lyo (-80ºC) W0		
Frozen (-80ºC) W24		
Lyo (-80ºC) W24		

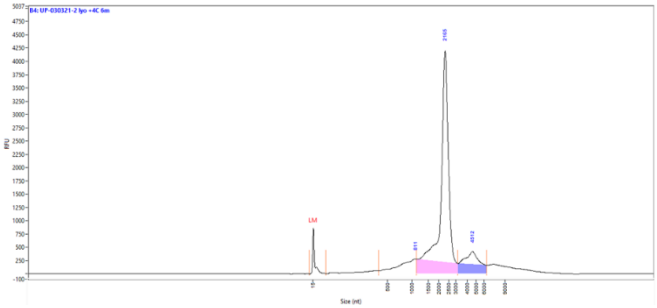
Lyo (-20ºC) W24



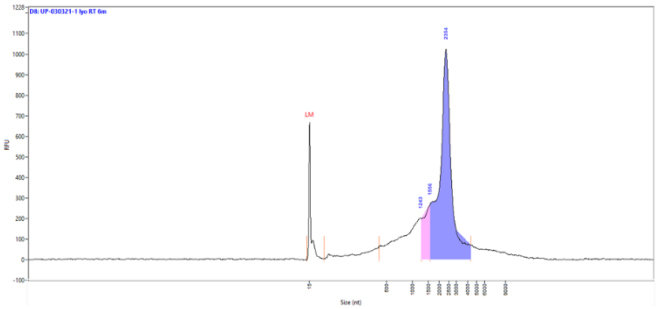


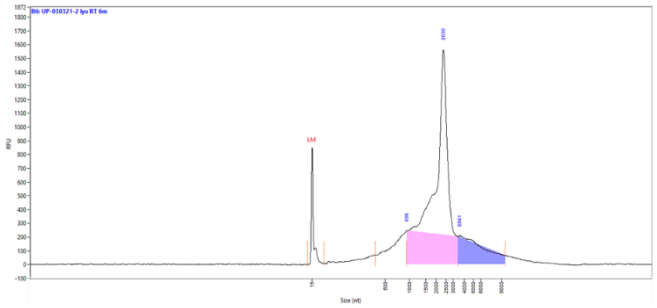
Lyo (4ºC) W24



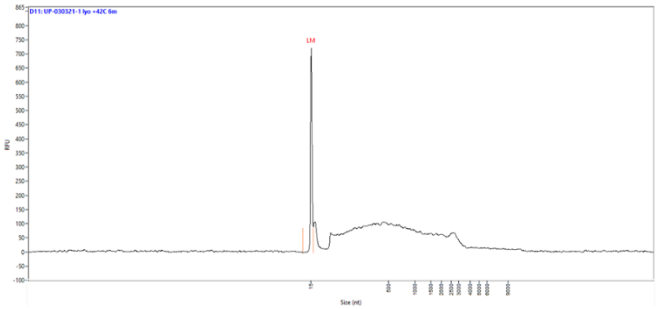


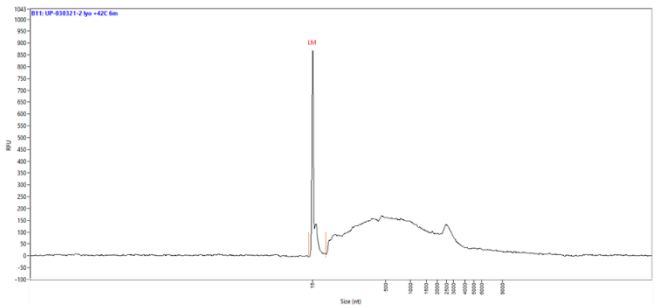
Lyo (25ºC) W24





Lyo (42ºC) W24





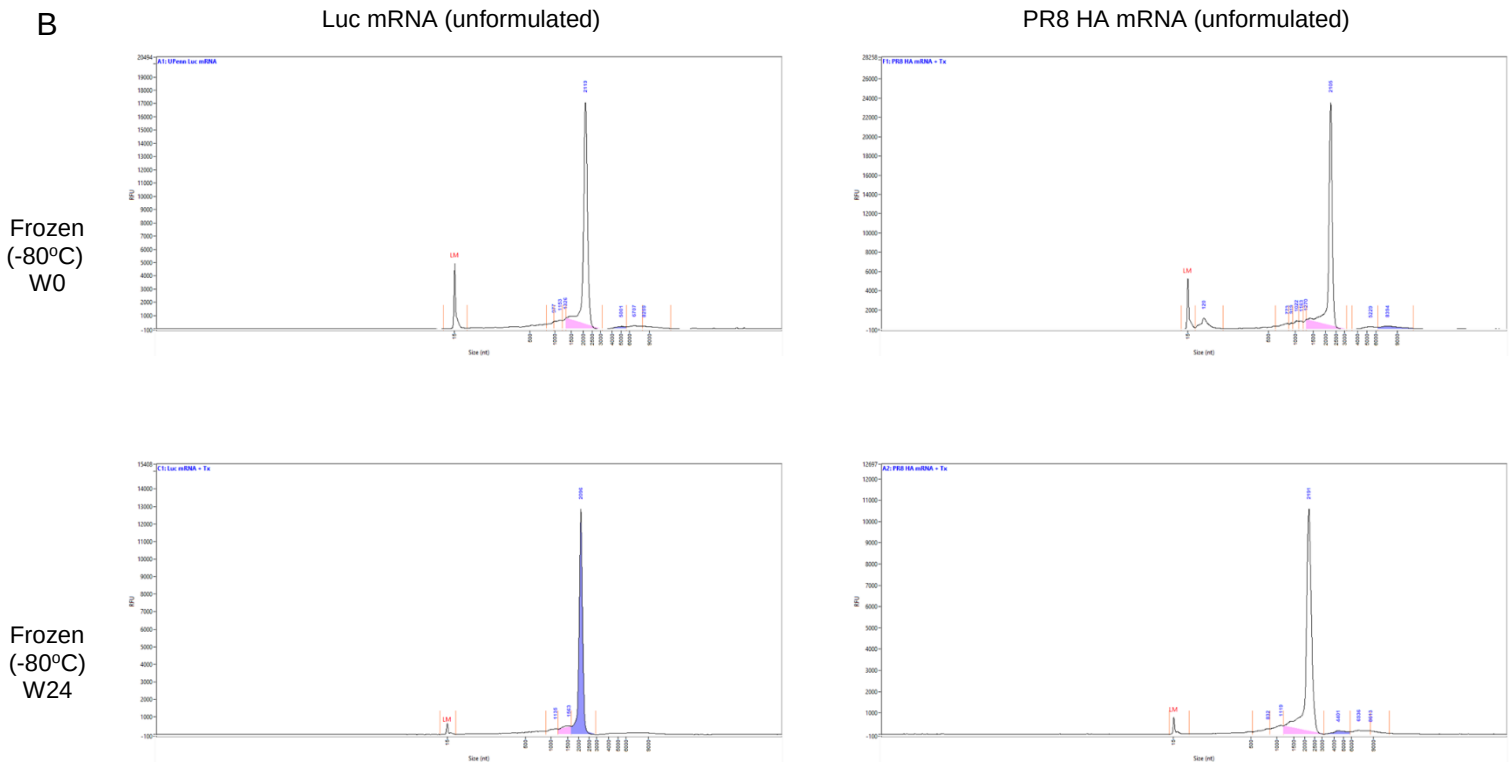


Figure S3. Related to Figure 1. Electropherograms of mRNA integrity analysis by capillary gel electrophoresis. All samples (unformulated mRNA and LNP) were treated with Triton X-100 to disrupt the particle and analyzed by capillary electrophoresis on the Agilent 5200 Fragment Analyzer. (A) mRNA integrity analysis post-thawing of frozen Luc and PR8 HA mRNA-LNPs stored at -80ºC and post-reconstitution of lyophilized Luc and PR8 HA mRNA-LNPs stored at -80ºC, -20ºC, 4ºC, 25ºC or 42ºC at week 0 and week 24. (B) mRNA integrity analysis of unformulated mRNA payloads at week 0 and week 24 stored at -80ºC.

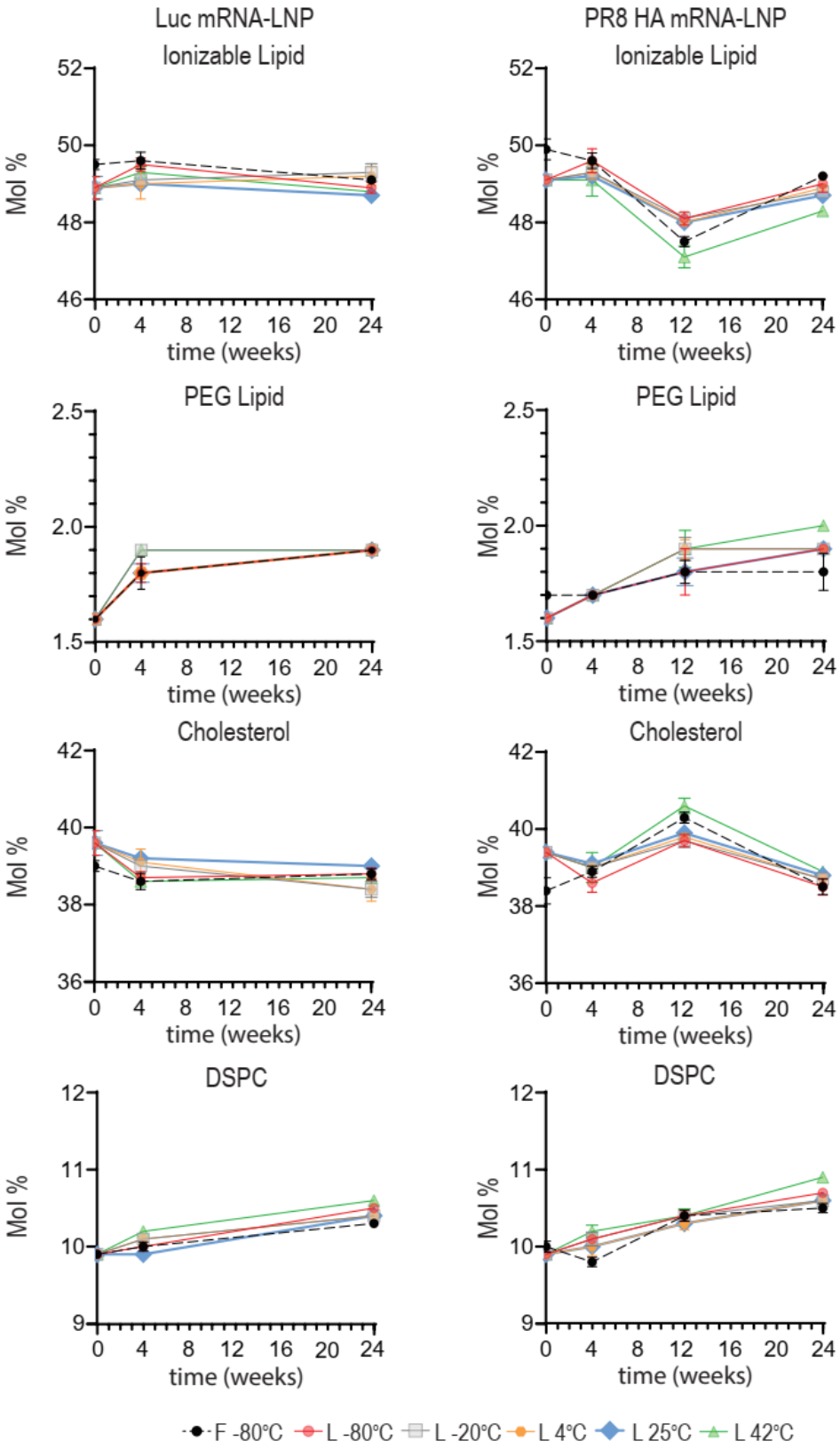
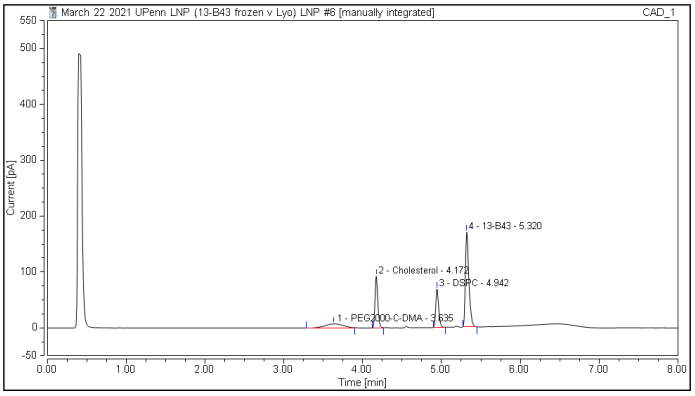
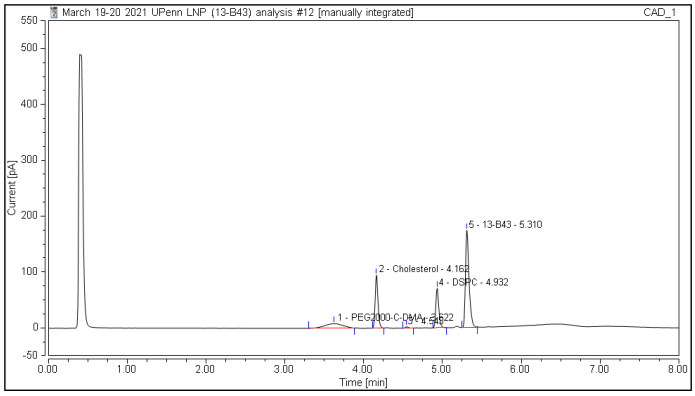
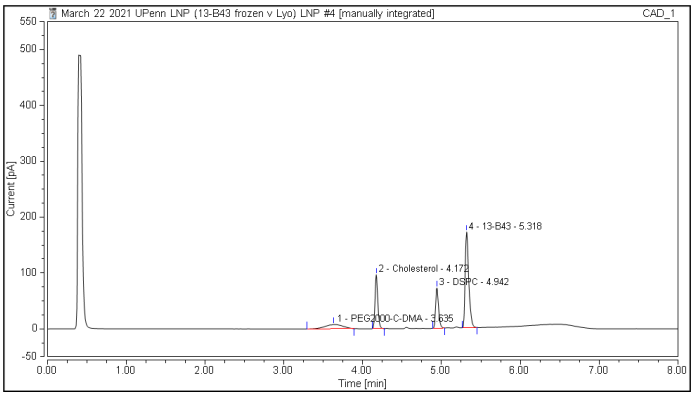
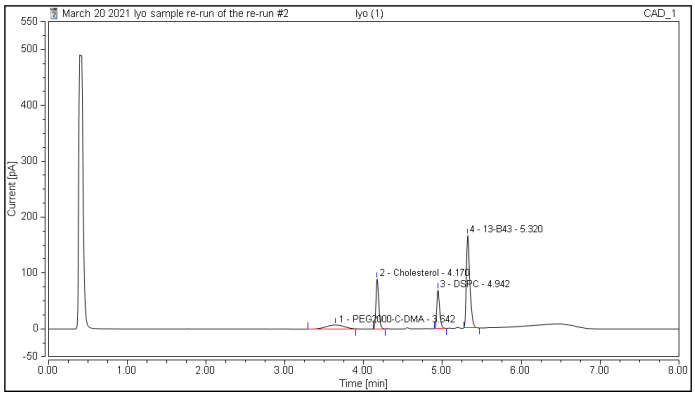
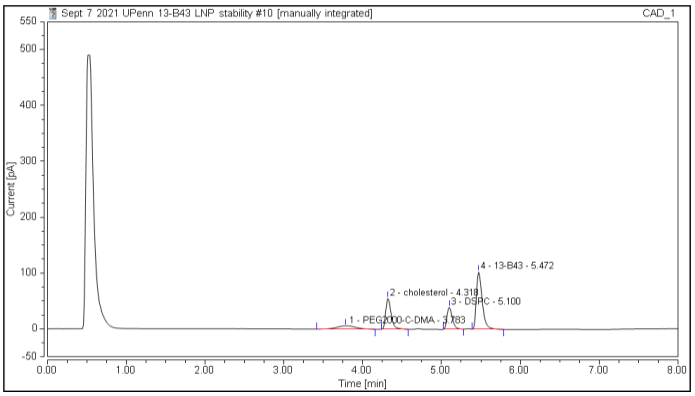
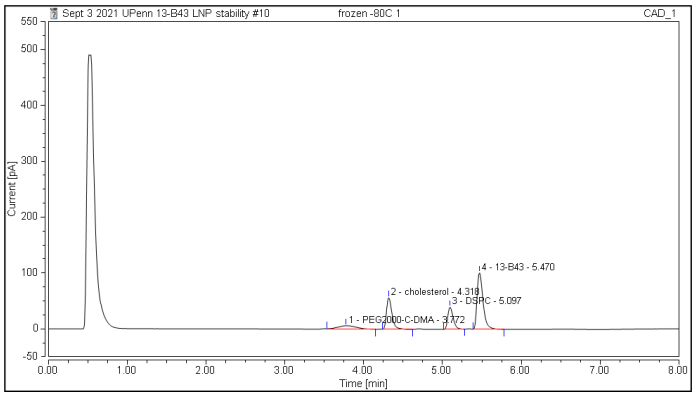
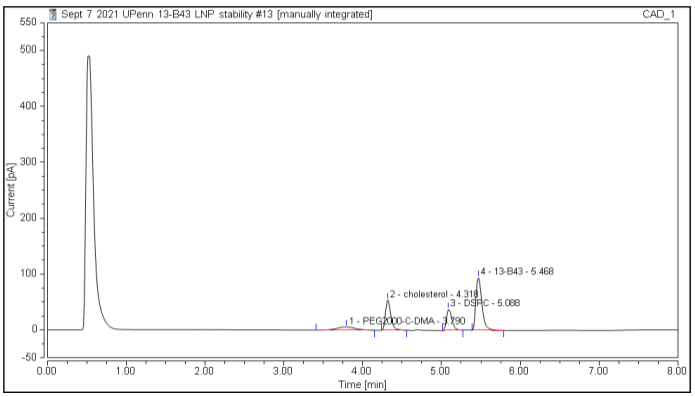
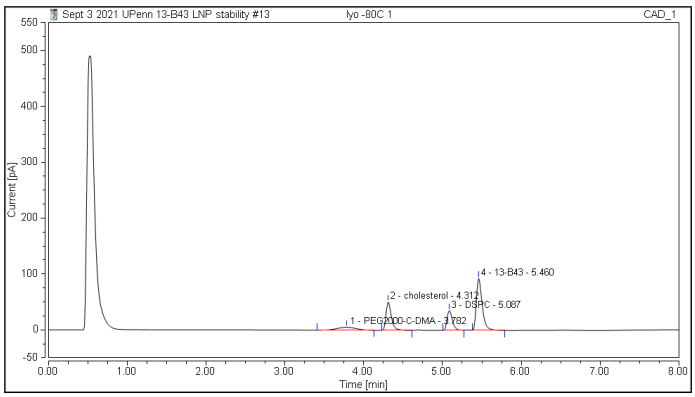


Figure S4. Related to Figure 1. UHPLC analysis of individual lipid components of Luc and PR8 HA mRNA-LNPs. UHPLC analysis post-thawing of frozen Luc and PR8 HA mRNA-LNPs stored at -80ºC and post-reconstitution of lyophilized Luc and PR8 HA mRNA-LNPs stored at -80ºC, -20ºC, 4ºC, 25ºC or 42ºC for 0, 4 or 24 (Luc) or 0, 4, 12, or 24 (PR8 HA) weeks. Analyses for all panels were done in triplicate. Error bars are SEM.

	Luc mRNA-LNP	PR8 HA mRNA-LNP
Frozen (-80ºC) W0		
Lyo (-80ºC) W0		
Frozen (-80ºC) W24		
Lyo (-80ºC) W24		

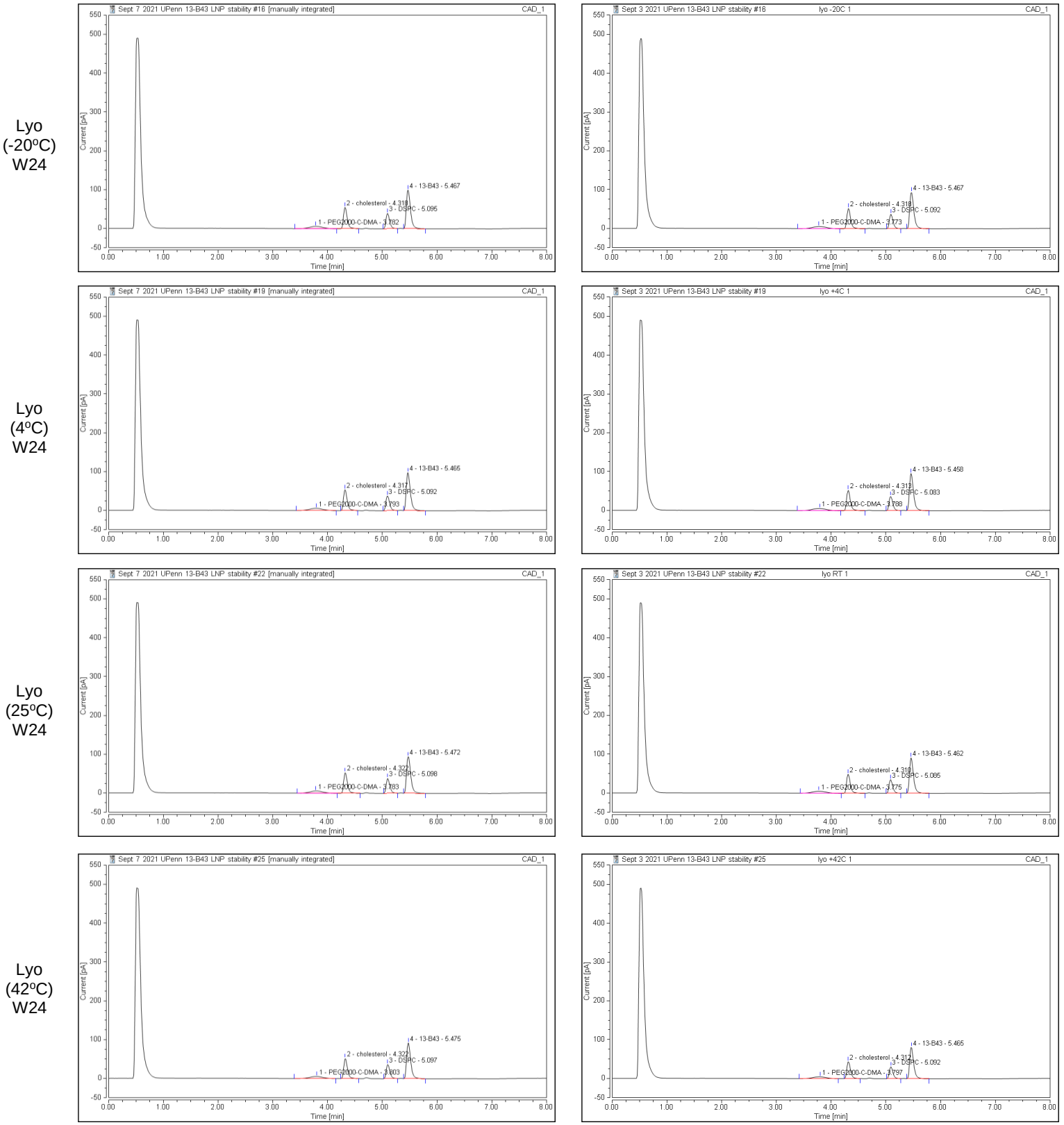


Figure S5. Related to Figure 1. UHPLC chromatograms of lipid components in Luc and PR8 HA mRNA-LNPs. UHPLC analysis post-thawing of frozen Luc and PR8 HA mRNA-LNPs stored at -80ºC and post-reconstitution of lyophilized Luc and PR8 HA mRNA-LNPs stored at -80ºC, -20ºC, 4ºC, 25ºC or 42ºC at week 0 and week 24. LNP samples were diluted to 1 mg/mL total lipid with ethanol and quantified against 5-point calibration curve for each of the components using the Thermo-Scientific Vanquish UHPLC system with a CAD detector.

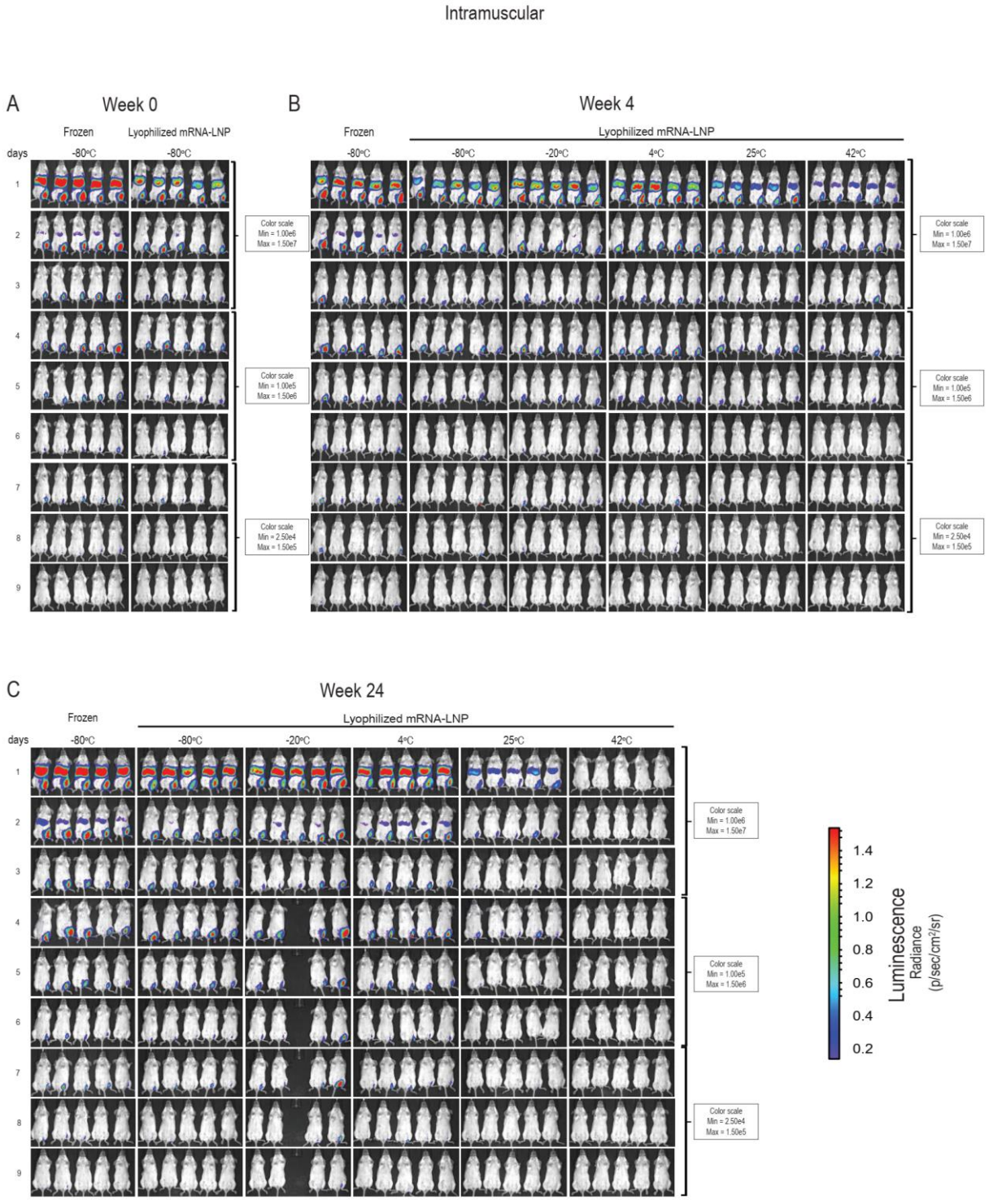


Figure S6. Related to Figure 2. In vivo imaging studies after intramuscular administration of Luc mRNA-LNPs. Representative IVIS images taken post (A) 0 weeks, (B) 4 weeks or (C) 24 weeks Luc mRNA-LNP injection. n= 5 mice per group. One animal died in the -20ºC week 24 group (panel C) from a non-treatment related reason 3 days after starting the bioluminescent measurement.

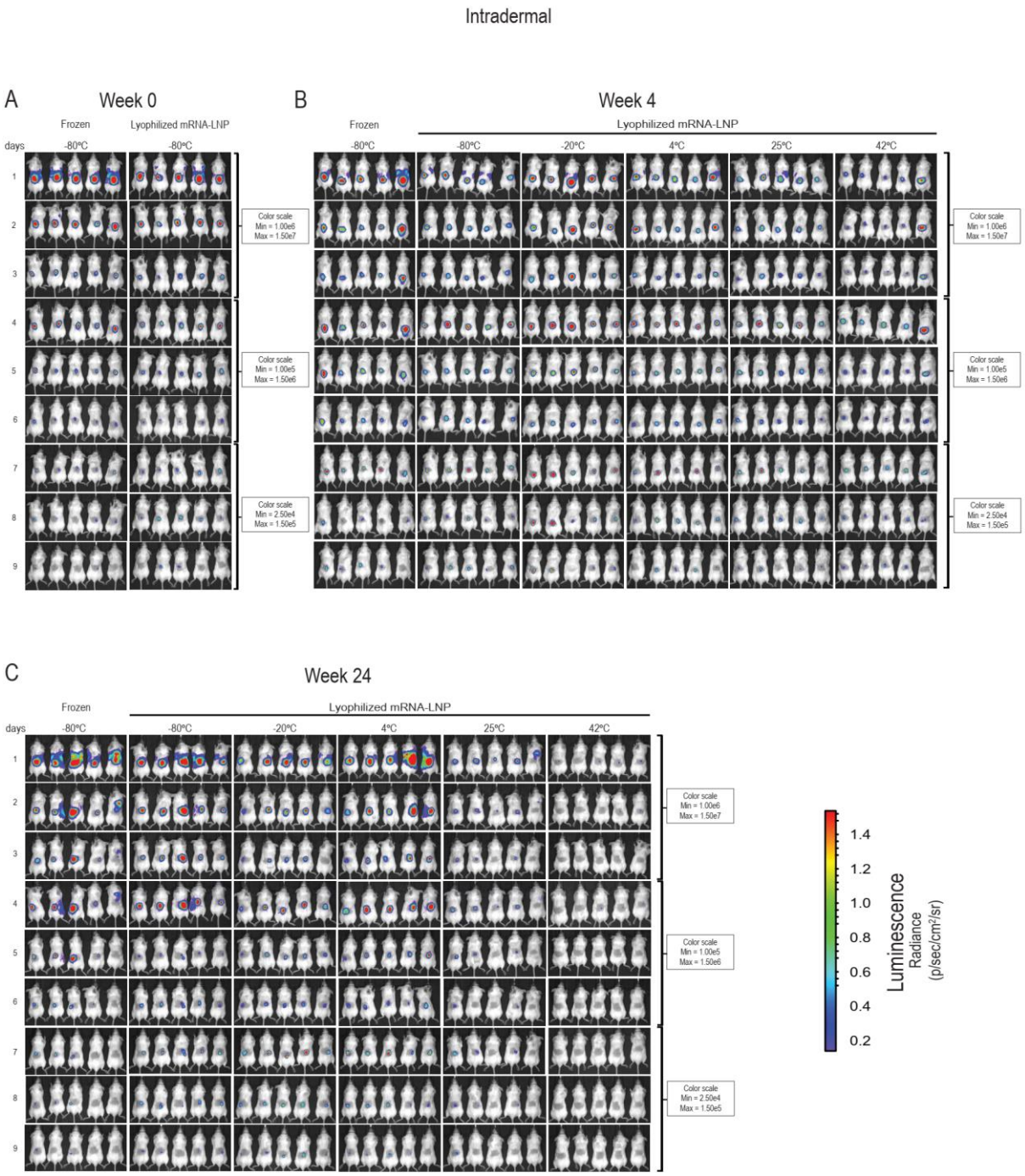


Figure S7. Related to Figure 2. In vivo imaging studies after intradermal administration of Luc mRNA-LNPs. Representative IVIS images taken post (A) 0 weeks, (B) 4 weeks or (C) 24 weeks Luc mRNA-LNP injection. n= 5 mice per group.

Time point	Z-average (nm)	Polydispersity	% Encapsulation
Release (T0)	73	0.08	99
1 year (-80ºC)	73	0.09	99

Table S1. Related to Figure 1. Stability of Luc mRNA-LNPs stored frozen at -80ºC. Particle size, polydispersity and encapsulation efficiency of frozen Luc mRNA-LNPs were measured after preparation (release, T0) and after storage at -80ºC for 1 year.

mRNA-LNP	Parameter	Wet	-80ºC frozen	Lyophilized /Reconstituted
Luc	Z-avg (nm)	77	80	97
	Polydispersity	0.02	0.08	0.06
	Encapsulation	97%	97%	93%
	[mRNA] mg/mL	0.51	0.48	0.46
PR8 HA	Z-avg (nm)	78	83	98
	Polydispersity	0.03	0.06	0.06
	Encapsulation	97%	97%	93%
	[mRNA] mg/mL	0.50	0.49	0.46

Table S2. Related to Figure 1. Physicochemical characterization of mRNA-LNPs before and after freezing or lyophilization/reconstitution at week 0. Wet formulation represents freshly prepared LNP, characterized post-sterile filtration, prior to freezing or lyophilization. Frozen sample represents LNP that was frozen at -80ºC and thawed at room temperature just prior to characterization. Lyophilized and reconstituted LNP represents a sample that was characterized following reconstitution of the lyophilized sample with water. Particle size and polydispersity were measured by dynamic light scattering. mRNA concentration and encapsulation were measured by RiboGreen assay.

Format	Parameter	-80ºC	-20ºC	4ºC	25ºC	42ºC
Non-lyophilized at week 4	Z-avg (nm)	81	128	89	85	86
	Polydispersity	0.06	0.08	0.16	0.15	0.15
	Encapsulation	97	92	97	97	97
	[mRNA] mg/mL	0.51	0.52	0.52	0.51	0.47
	mRNA integrity (%)	101	106	104	89	12
Lyophilized at week 4	Z-avg (nm)	97	95	95	97	137
	Polydispersity	0.05	0.06	0.06	0.07	0.08
	Encapsulation	92	93	93	93	91
	[mRNA] mg/mL	0.47	0.46	0.45	0.46	0.42
	mRNA integrity (%)	104	104	101	92	36

Table S3. Related to Figure 1. Physicochemical characterization of Luc mRNA-LNPs in non-lyophilized versus lyophilized format after 4 weeks of storage at the specified temperatures. Non-lyophilized and lyophilized Luc mRNA-LNPs were characterized following 4 weeks of storage at -80ºC, -20ºC, 4ºC, 25ºC, and 42ºC. Particle size and polydispersity were measured by dynamic light scattering, encapsulation and mRNA concentration were measured by the RiboGreen assay, and mRNA integrity was determined by capillary electrophoresis.