Supporting Information

Lipid Nanoparticle–Assisted mRNA Delivery for Potent Cancer Immunotherapy

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Figure S1. Correlation of the obtained CD 8 T cell levels on the molar percentage of cKK-E12 in formulations evaluated in Library B. The T cell levels seem to increase upon lowering the percentage of cKK-E12.

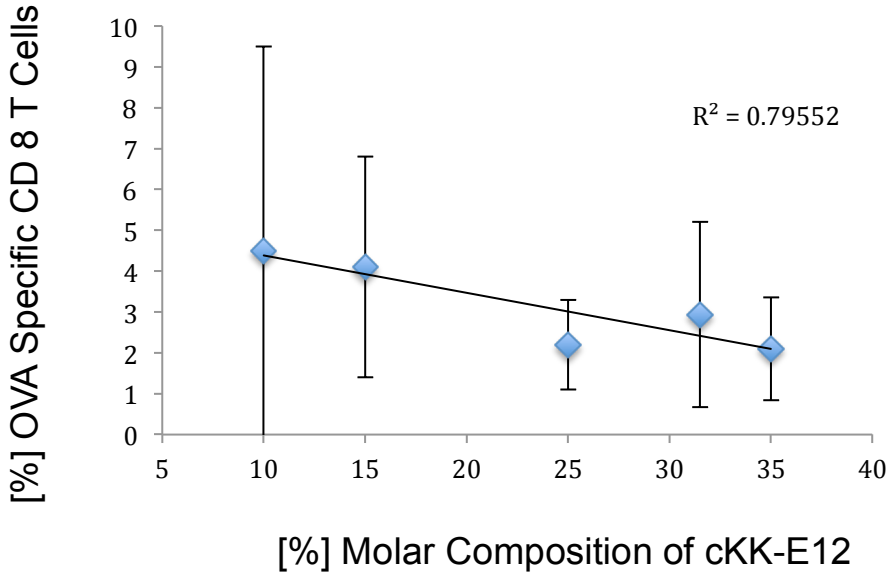


Table S1. Parameters for the preparation of LNP formulations used in this study, including characterization and efficacy of Libraries A and B for elucidating an antigen specific Cd 8 T cell response in mice *in vivo*.

Code	A	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8
ionizable lipid/mRNA	10	10	10	10	10	10	10	10	10
ionizable lipid	C12-200	C12-200	cKK-E12	503O13	DOTAP	DODAP	C12-200	C12-200	C12-200
Mol %	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5
phospho lipid	DOPE	DOPE	DOPE	DOPE	DOPE	DOPE	DSPC	DOTAP	POPE
Mol %	10	0	10	10	10	10	10	10	10
cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol
Mol %	36	36	36	36	36	36	36	36	36
PEG lipid	C14-PEG1000	C14-PEG1000	C14-PEG1000	C14-PEG1000	C14-PEG1000	C14-PEG1000	C14-PEG1000	C14-PEG1000	C14-PEG1000
Mol %	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
additive	Arachidonic acid	Arachidonic acid	Arachidonic acid	Arachidonic acid	Arachidonic acid	Arachidonic acid	Arachidonic acid	Arachidonic acid	Arachidonic acid
Mol %	20	30	20	20	20	20	20	20	20
Peak 1 (nm)	121	129	76.35	82.15	114.1	218.6	89.29	125.9	123.9
Peak 1 (%)	97.3	98.6	100	91	96.6	54	100	98.6	96.2
PDI	0.388	0.173	0.101	0.254	0.304	0.429	0.126	0.147	0.342
%entrapment	83.31	47.00	93.05	94.92	78.24	96.97	92.54	86.54	96.32
%-OVA specific CD8 (@day 7)	1.14	0.42	2.94	0.98	0.16	0.2	3.12	0.92	1.55
Standard deviation	1.29	0.28	2.27	1.41	0.06	0.13	1.58	0.84	1.30

Code	A-9	A-10	A-11	A-12	A-13	A-14	A-15	A-16	A-17
ionizable lipid/mRNA	10	10	10	10	10	10	10	10	10
ionizable lipid	C12-200	C12-200	C12-200	C12-200	C12-200	C12-200	C12-200	C12-200	C12-200
Mol %	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5
phospho lipid	DMPC	DOPS	DOPE	DOPE	DOPE	DOPE	DOPE	DOPE	DOPE
Mol %	10	10	10	10	10	10	10	10	10
cholesterol	Cholesterol	Cholesterol	DC-cholester	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol
Mol %	36	36	36	36	36	36	36	36	36
PEG lipid	C14-PEG1000	C14-PEG1000	C14-PEG1000	C14-PEG2000	C14-PEG350	C14-PEG3000	C18-PEG2000	C14-PEG1000	C14-PEG1000
Mol %	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
additive	Arachidonic acid	Arachidonic acid	Arachidonic acid	Arachidonic acid	Arachidonic acid	Arachidonic acid	Arachidonic acid	Oleic Acid	Myristic acid
Mol %	20	20	20	20	20	20	20	20	20
Peak 1 (nm)	63.7	79	69.11	67.53	232.4	96.4	110	64.43	77.66
Peak 1 (%)	100	95	100	100	100	100	72	98.2	94.6
PDI	0.172	0.228	0.18	0.161	0.144	0.182	0.297	0.166	0.227
%entrapment	88.41	92.70	87.54	52.51	62.09	64.99	84.90	80.76	89.28
%-OVA specific CD8 (@day 7)	0.9	2.8	0.84	2	0.14	1.2	0.5	1.64	0.98
Standard deviation	0.73	1.69	1.43	2.21	0.11	0.40	0.27	1.77	0.68

Code	A-18	A-19	B-1	B-2	B-3	B-4	B-5	B-6	B-7
ionizable lipid/mRNA	10	10	10	10	10	10	10	5	10
ionizable lipid	C12-200	C12-200	cKK-E12	cKK-E12	cKK-E12	cKK-E12	cKK-E12	cKK-E12	cKK-E12
Mol %	31.5	35	35	35	32.5	25	25	35	25
phospho lipid	DOPE	DOPE	DOPE	DOPS	DOPE	DOPE	DOPE	DOPE	DOPS
Mol %	10	16	16	16	7.5	26	37.5	16	37.5
cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol
Mol %	36	46.5	46.5	46.5	43	46.5	35	46.5	35
PEG lipid	C14-PEG1000	C14-PEG1000	C14-PEG2000	C14-PEG2000	C14-PEG2000	C14-PEG2000	C14-PEG2000	C14-PEG2000	C14-PEG2000
Mol %	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
additive	Sodium Lauryl Sulfate	Arachidonic acid	Arachidonic acid	Arachidonic acid	Sodium Lauryl Sulfate	Arachidonic acid	Arachidonic acid	Arachidonic acid	Arachidonic acid
Mol %	20	0	0	0	14.5	0	0	0	0
Peak 1 (nm)	108	41.38	120	101	80.3	86.6	104	103	
Peak 1 (%)	100	60	97	97	97	99	99	97	
PDI	0.166	0.299	0.266	0.283	0.192	0.25	0.223	0.258	
%entrapment	87.10	59.79	81.60	90.00	82.10	87.20	91.30	88.80	58.73
%-OVA specific CD8 (@day 7)	3.1	2.86	2.1	4.1	2.1	2.2	3.7	1.8	0.5
Standard deviation	0.75	2.54	1.26	2.80	0.98	1.10	3.48	1.68	0.17

Code	B-8	B-9	B-10	B-11	B-12	B-13
ionizable lipid/mRNA	10	10	5	10	10	10
ionizable lipid	cKK-E12	cKK-E12	cKK-E12	cKK-E12	cKK-E12	cKK-E12
Mol %	15	15	15	15	10	15
phospho lipid	DOPE	DOPE	DOPE	DOPE	DOPE	DOPS
Mol %	26	47.5	47.5	26	26	26
cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol	Cholesterol
Mol %	56.5	35	35	40.5	61.5	56.5
PEG lipid	C14-PEG2000	C14-PEG2000	C14-PEG2000	C14-PEG2000	C14-PEG2000	C14-PEG2000
Mol %	2.5	2.5	2.5	2.5	2.5	2.5
additive	Arachidonic acid	Arachidonic acid	Arachidonic acid	Sodium Lauryl Sulfate	Arachidonic acid	Sodium Lauryl Sulfate
Mol %	0	0	0	16	0	0
Peak 1 (nm)	75.15	93.62	57.21	152.2	121.3	111.1
Peak 1 (%)	96.4	100	60	100	100	97.7
PDI	0.229	0.291	0.326	0.217	0.241	0.243
%entrapment	90.90	85.17	64.21	84.07	91.96	47.47
%-OVA specific CD8 (@day 7)	4.1	2.7	1.9	4.2	4.5	1.7
Standard deviation	2.70	1.60	1.60	1.50	5.00	0.52

Figure S2. Only low levels of OVA specific IgG antibodies were detected 7 weeks after a single immunization. At dilutions of 1:16 or smaller, the OVA specific IgG titers of treated mice were more than a standard deviation from the control group, mice treated with irrelevant mRNA containing LNP. The irrelevant mRNA used for this study was coding for Beta galactosidase.

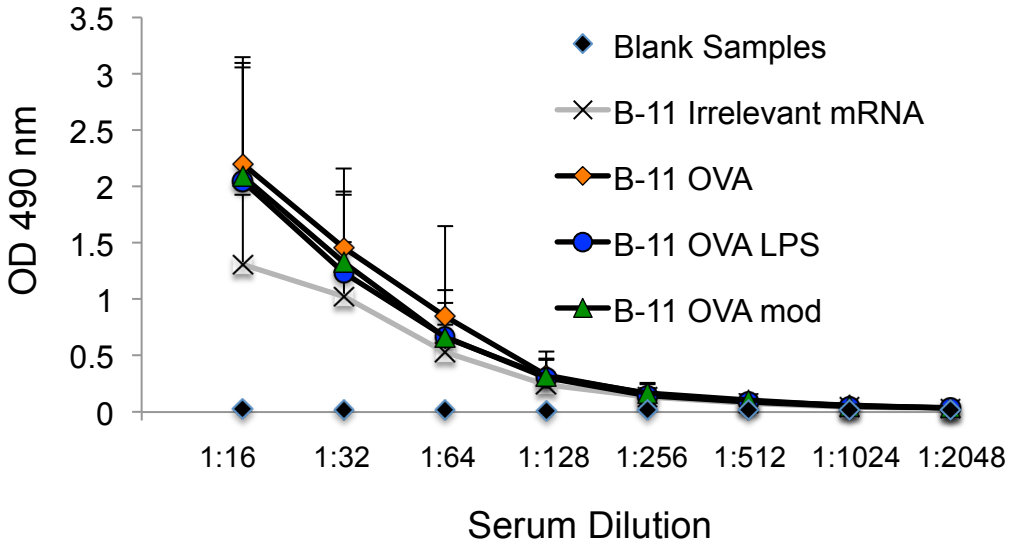
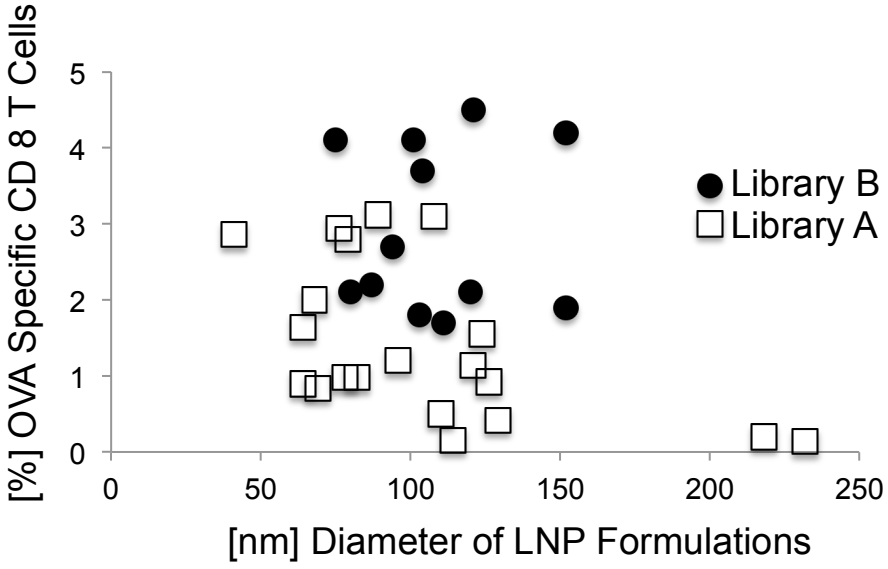
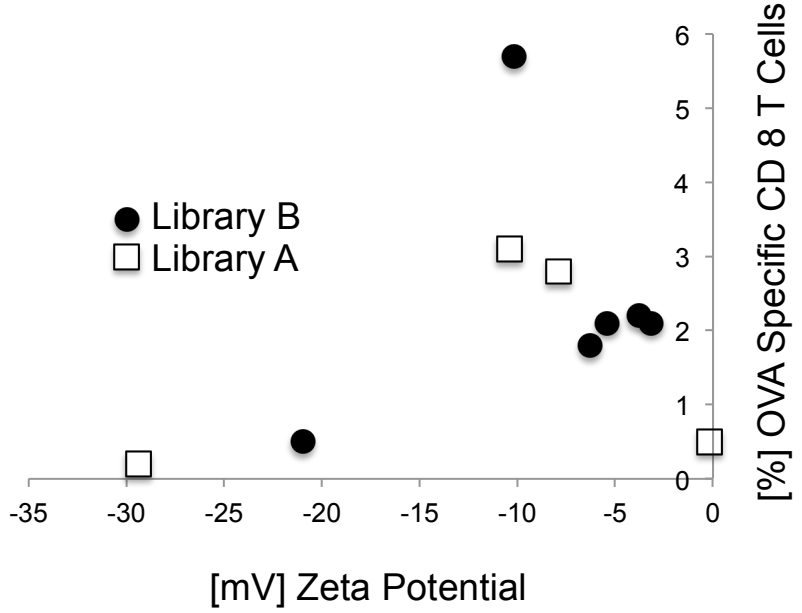


Figure S3. Among the formulations tested (Table S1) there was no correlation between: Particle size and CD 8 T cell expansion (A), Zeta potential and CD 8 T cell expansion (B), and Zeta potential and Z-average. Each dot represents an entry from the formulation optimization (Table S1).

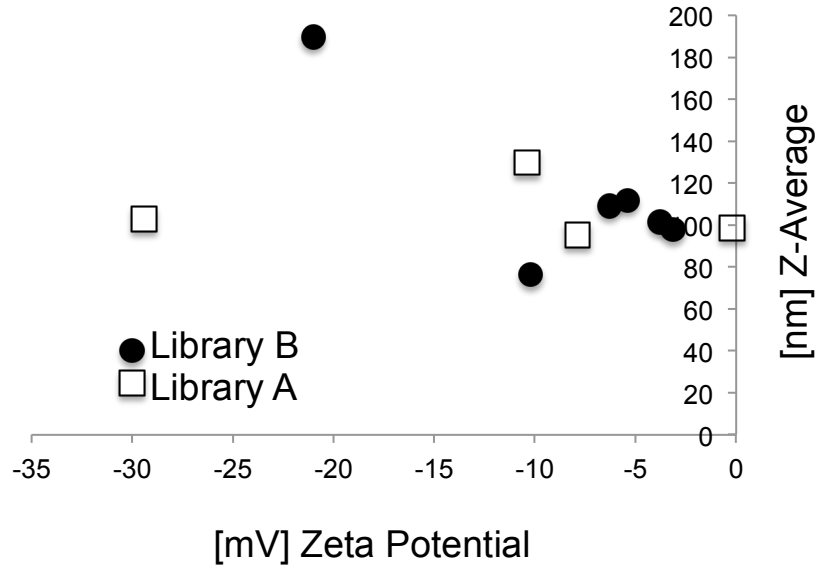
A)



B)


Library B
Library A
6
5
4
3
2
1
0
-35
-30
-25
-20
-15
-10
-5
0
[%] OVA Specific CD 8 T Cells
[mV] Zeta Potential

C)


Library B
Library A
200
180
160
140
120
100
80
60
40
20
0
-35
-30
-25
-20
-15
-10
-5
0
[nm] Z-Average
[mV] Zeta Potential

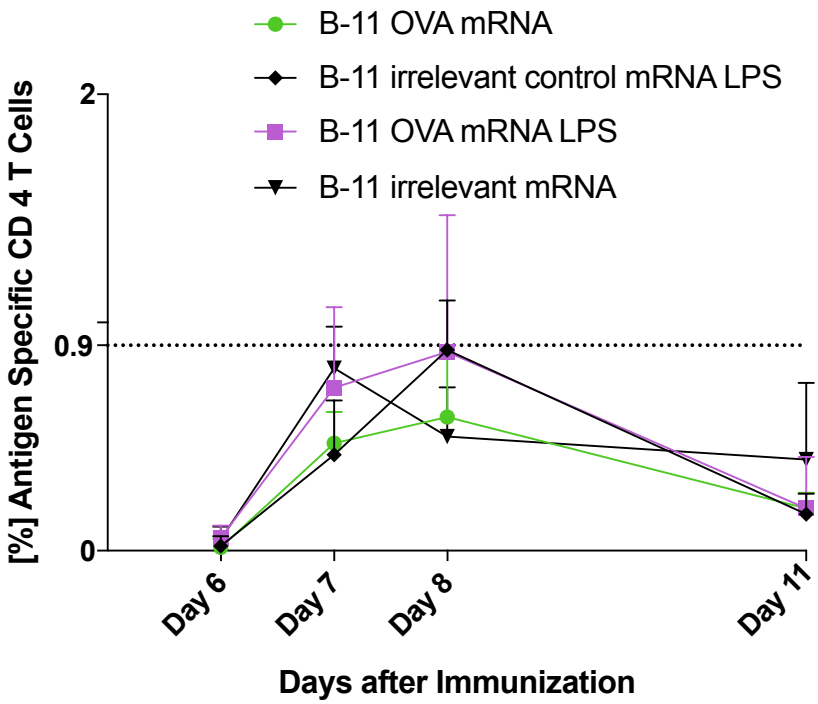


Figure S4. Percent circulating, antigen specific CD 4 T cells in the peripheral blood. The groups are not statistically significant different. LNP containing mRNA coding for β-Galactosidase was used as irrelevant control.