Supporting Information

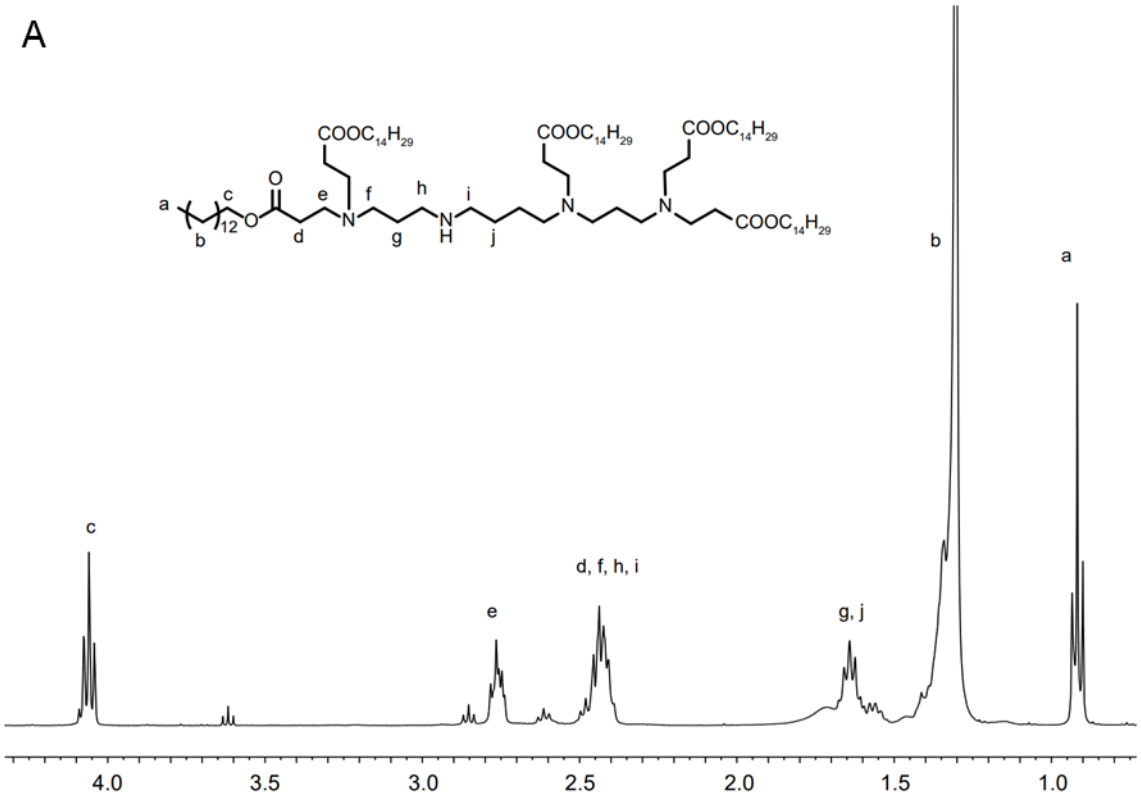
Boosting Ionizable Lipid Nanoparticle-mediated In Vivo mRNA Delivery through Optimization of Lipid Amine-Head Group

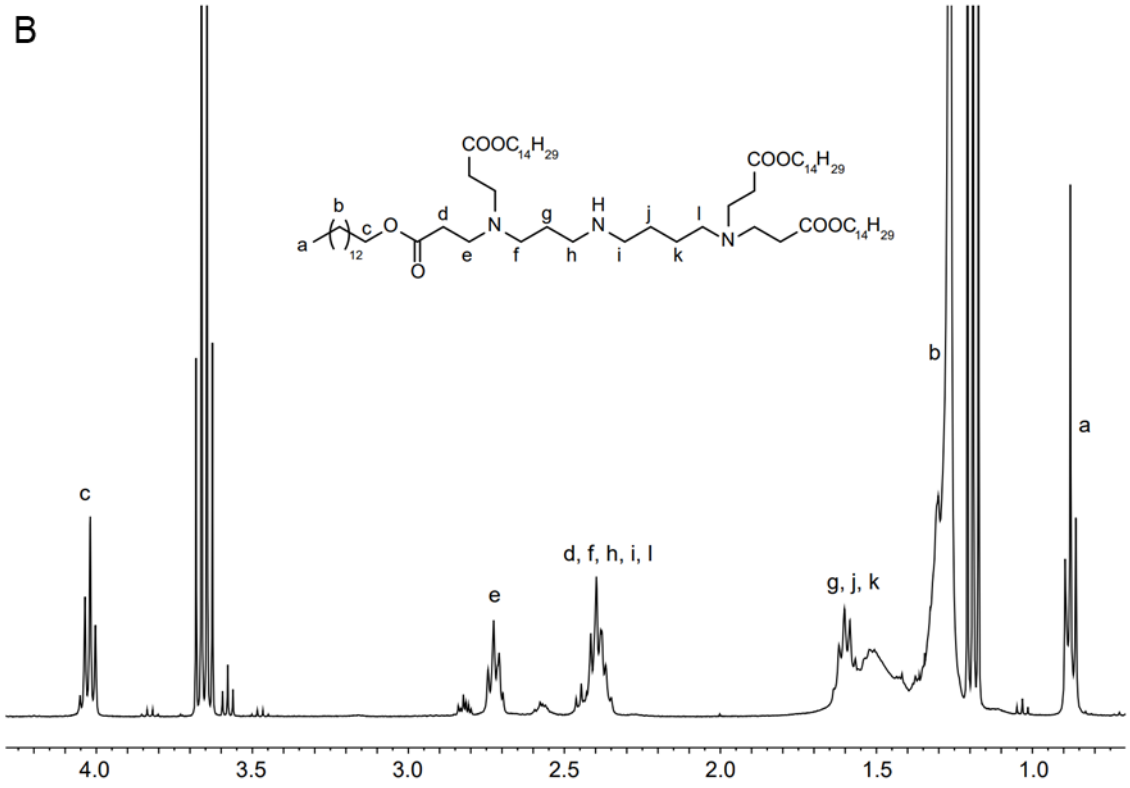
*Feng Ding, Hongqian Zhang, Jiwei Cui, Qiang Li, Chuanxu Yang**

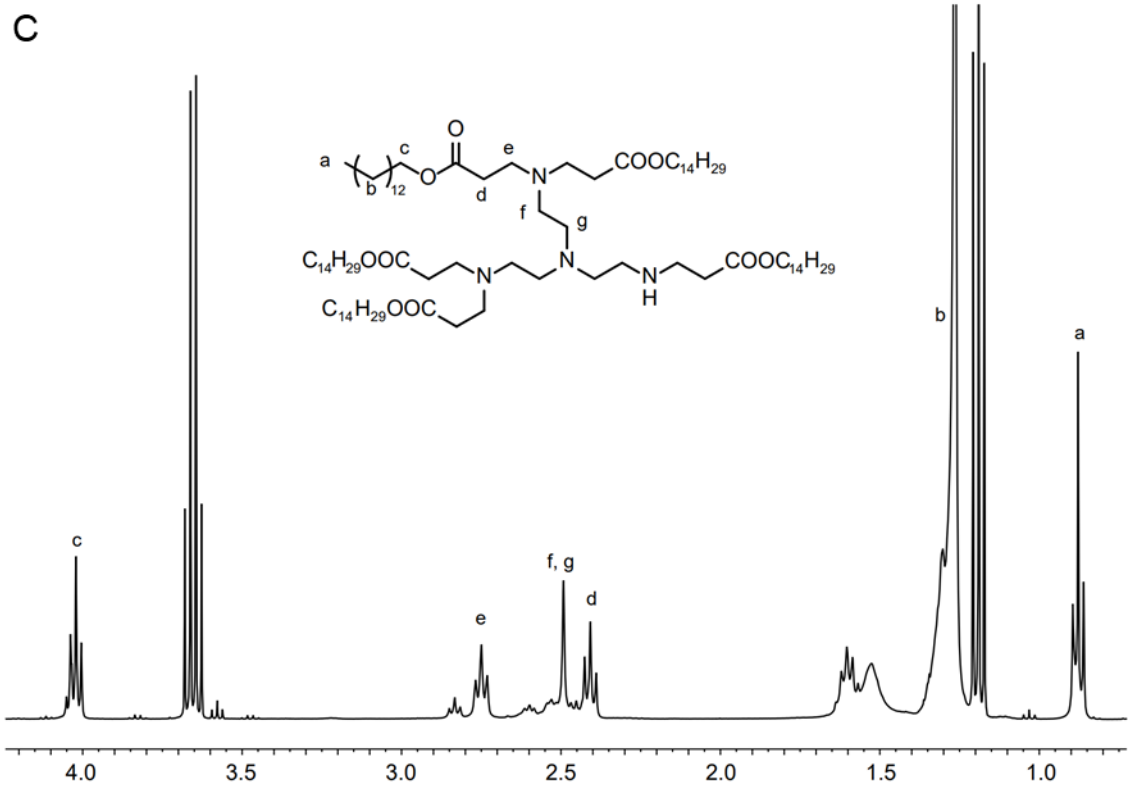
Key Laboratory of Colloid and Interface Chemistry of the Ministry of Education, and School of Chemistry and Chemical Engineering, Shandong University, Jinan 25010, China

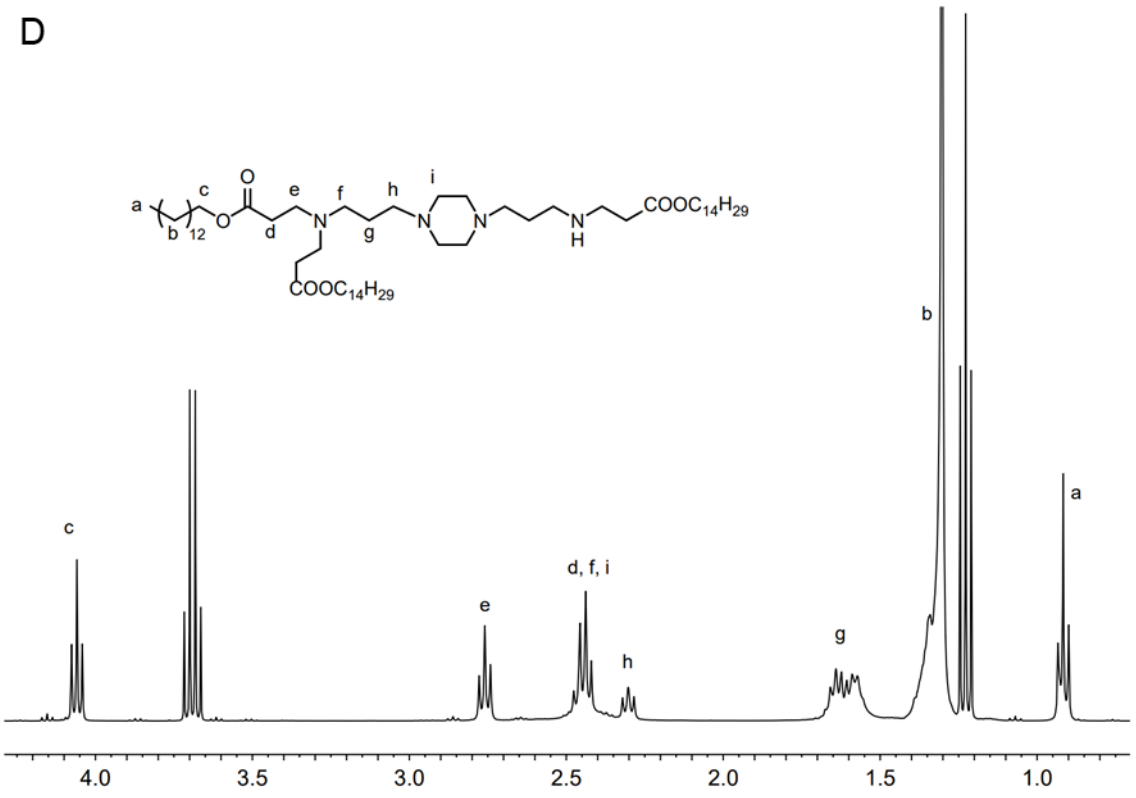
Table S1. Primers used for RT-qPCR.

Gene	Forward (5'-3')	Reverse (5'-3')
GAPDH	GACGGCCGCATCTTCTTGTG	GCGCCCAATACGGCCAAATC
EPO	TACCTGCTGGAGGCCAA	CACGGTGATGTTCTCGTTCA


A
COOC14H29
COOC14H29
COOC14H29
COOC14H29
a
b
c
d
e
f
g
h
i
j
12
N
H
O
b
a
c
d, f, h, i
e
g, j
4.0
3.5
3.0
2.5
2.0
1.5
1.0


B
COOC14H29
COOC14H29
COOC14H29
a
b
c
d
e
f
g
h
i
j
k
l
12
N
H
O
b
a
c
d, f, h, i, l
e
g, j, k
4.0
3.5
3.0
2.5
2.0
1.5
1.0


C
a
b
12
c
O
O
d
e
N
COOC14H29
f
g
C14H29OOC
N
N
N
H
COOC14H29
C14H29OOC
b
a
c
f, g
d
e
4.0
3.5
3.0
2.5
2.0
1.5
1.0


D
a
b
12
c
O
O
d
e
N
f
g
h
i
N
N
N
H
COOC14H29
COOC14H29
b
a
c
d, f, i
e
g
h
4.0
3.5
3.0
2.5
2.0
1.5
1.0

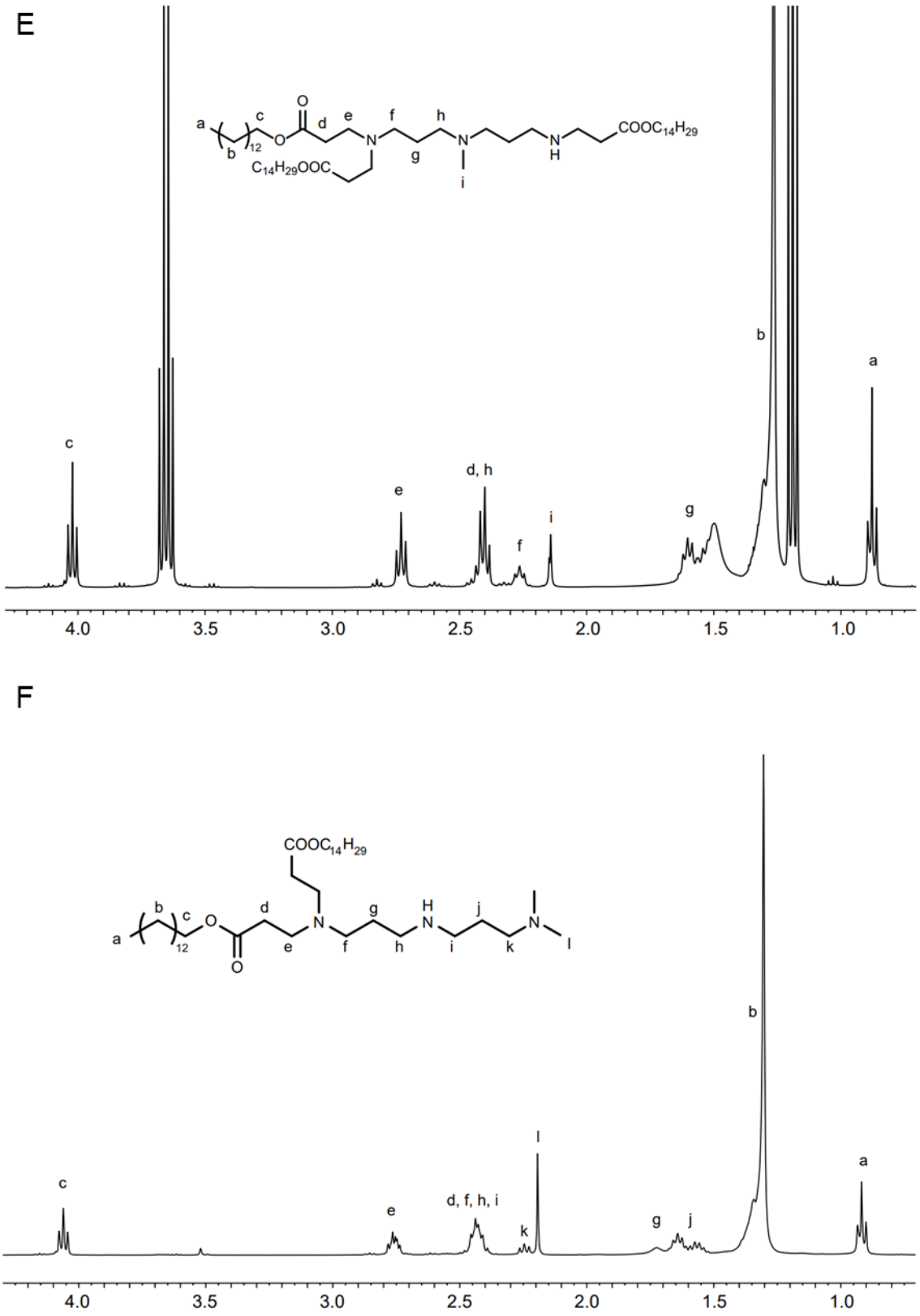


Figure S1. A representative ^{1}H NMR spectrum of 114 (A), 214 (B), 314 (C), 414 (D), 514 (E), 614 (F) ionizable lipids in CD_2Cl_2.

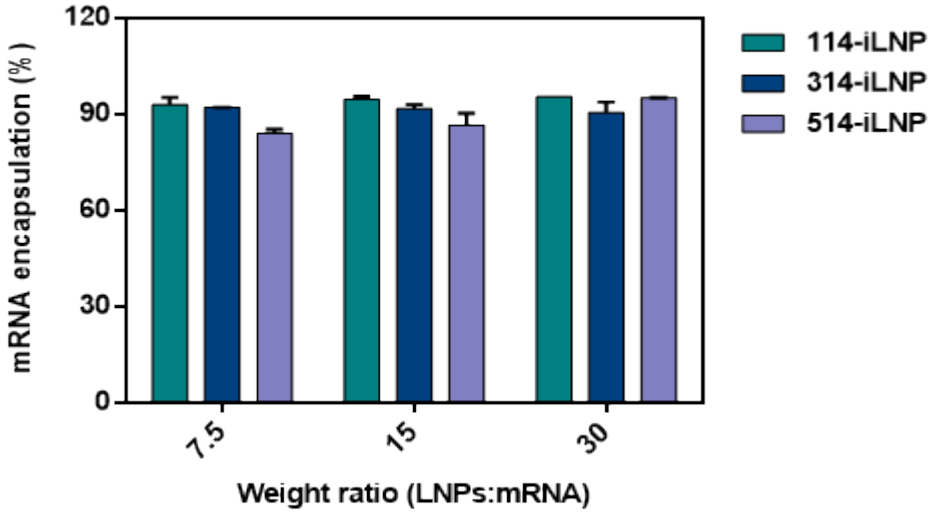


Figure S2. The mRNA encapsulation determined by RiboGreen assay at different weight ratio of iLNP:mRNA (n=2).

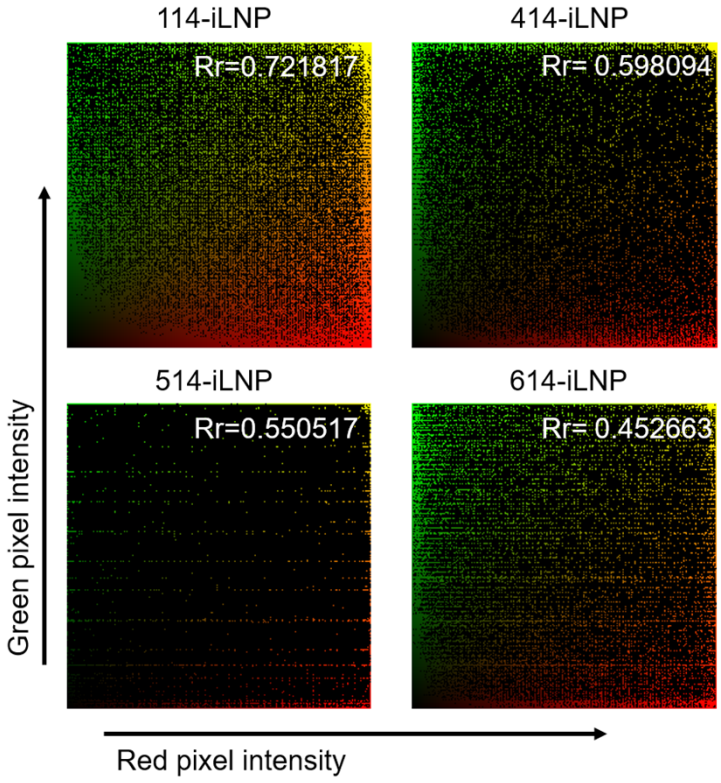


Figure S3. Colocalization analysis of endosomal escaping from iLNP/mRNA by Pearson's correlation.

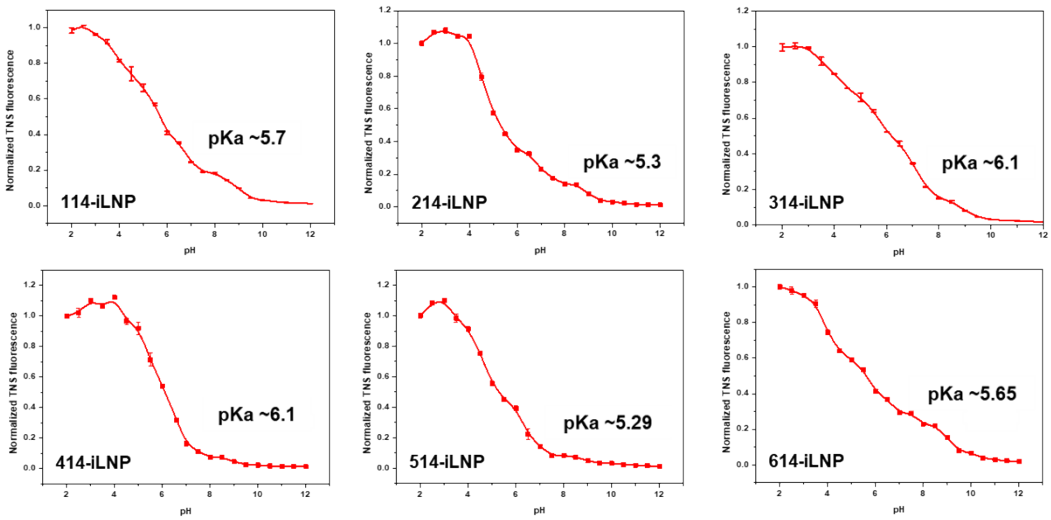


Figure S4. The pKa of iLNPs determined by TNS assay (n=2).

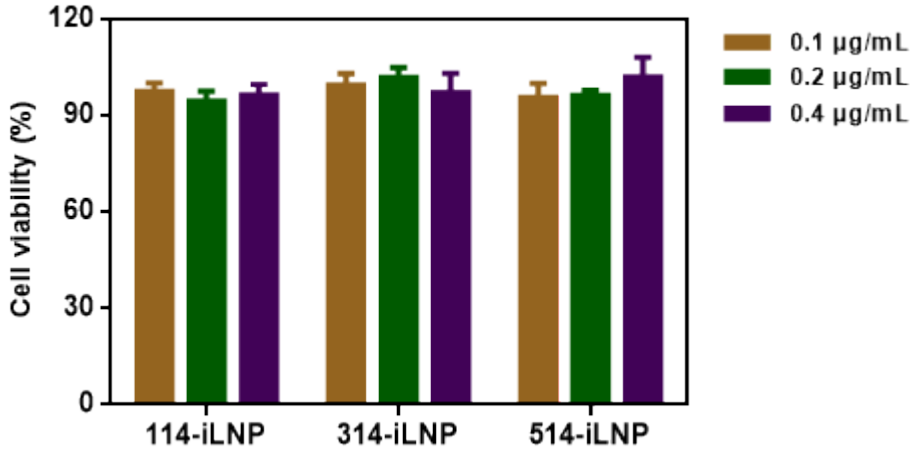


Figure S5. Cell viability of HeLa cells treated with top performance iLNP/mRNA complexes at mRNA concentration of 0.1, 0.2 or 0.4 μg/mL RNA for 24 h (n=3).

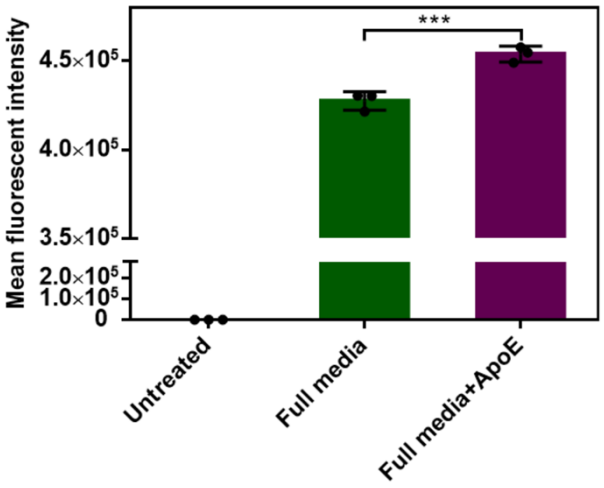


Figure S6. Quantification of cellular uptake in HeLa cells following treatment of cells with 114-iLNP in the absence or presence of Apolipoprotein (ApoE, 1 μg/mL) (n=3). Statistical analysis was performed using One-way ANOVA with Tukey test (GraphPad software Prism, Version 6.01) and statistical difference was defined as ***$p < 0.001$.

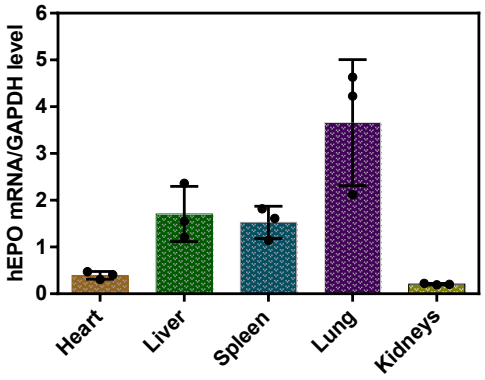


Figure S7. Quantification of hEPO mRNA in major organs by RT-qPCR including heart, liver, spleen, lung and kidneys after 6h i.v. injection of 114-iLNP/mhEPO at mRNA dose of 0.5 mg/kg (n=3). GAPDH was utilized as house-keeping gene during RT-qPCR analysis.