Supporting Information for "A potent branched-tail lipid nanoparticle enables multiplexed mRNA delivery and gene editing in vivo"

Khalid A. Hajj [a,*], Jilian R. Melamed [a,*], Namit Chaudhary [a], Nicholas G. Lamson [a], Rebecca L. Ball [a], Saigopalakrishna S. Yerneni [b], and Kathryn A. Whitehead [a,b,†]

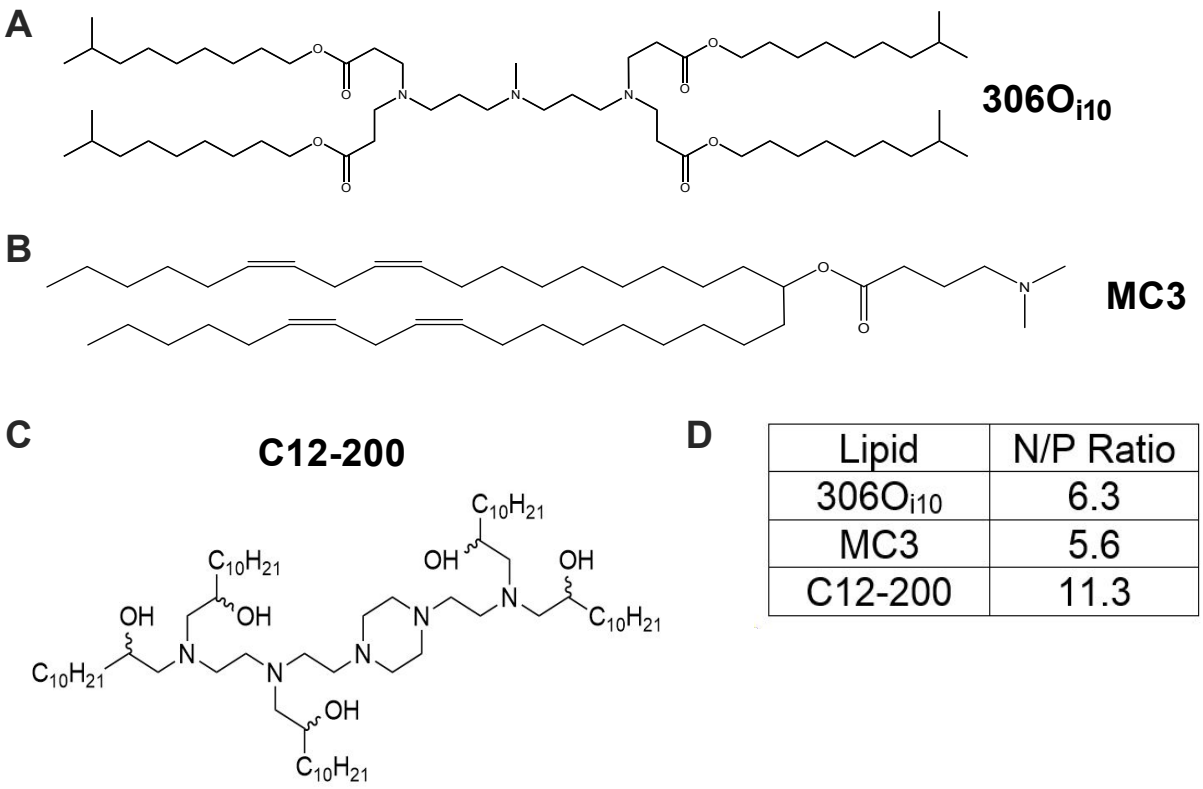
[a] Department of Chemical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213

[b] Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213

*Authors contributed equally

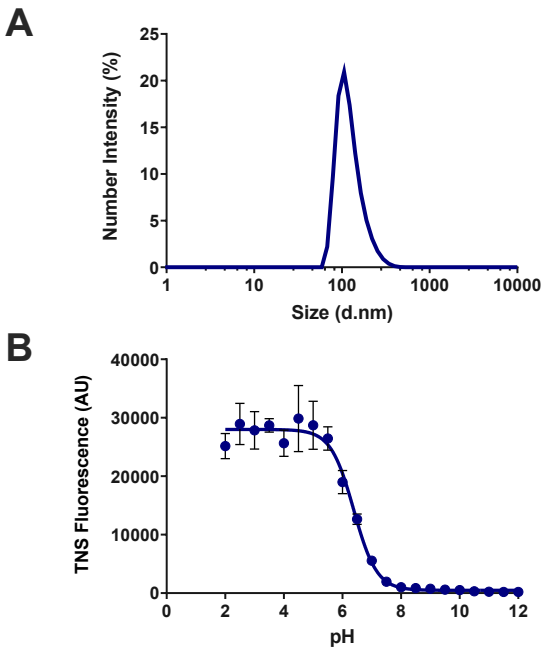
[†]Correspondence should be addressed to K.A.W.: kawhite@cmu.edu

Keywords: mRNA delivery, lipidoid, lipid nanoparticles, gene editing, protein expression, liver delivery



Lipid	N/P Ratio
306O$_{i10}$	6.3
MC3	5.6
C12-200	11.3

Figure S1. Structures of lipids used in this work. The structures of the lipids (A) 306O$_{i10}$, (B) MC3, and (C) C12-200 are shown here. (D) N/P ratios used to formulate lipids into mRNA lipid nanoparticles.



C

Size (nm)	124.6
PDI	0.12
Zeta Potential (mV)	0.43
pKa	6.4
mRNA Entrapment (%)	91.1

Figure S2. The lipidoid $306O_{i10}$ is a potent, ionizable lipid-like material. (A) $306O_{i10}$ nanoparticles had a number average diameter of 125 nm with a PDI of 0.12, as measured by DLS. (B) The pKa of $306O_{i10}$ nanoparticles was 6.4, as measured by the TNS assay. The pKa is the pH at which the TNS fluorescence value is half of the maximum value. Error bars represent s.d. (n = 5). (C) Nanoparticles also had a neutral zeta potential at pH 7.4, and an mRNA entrapment efficiency of 91%.

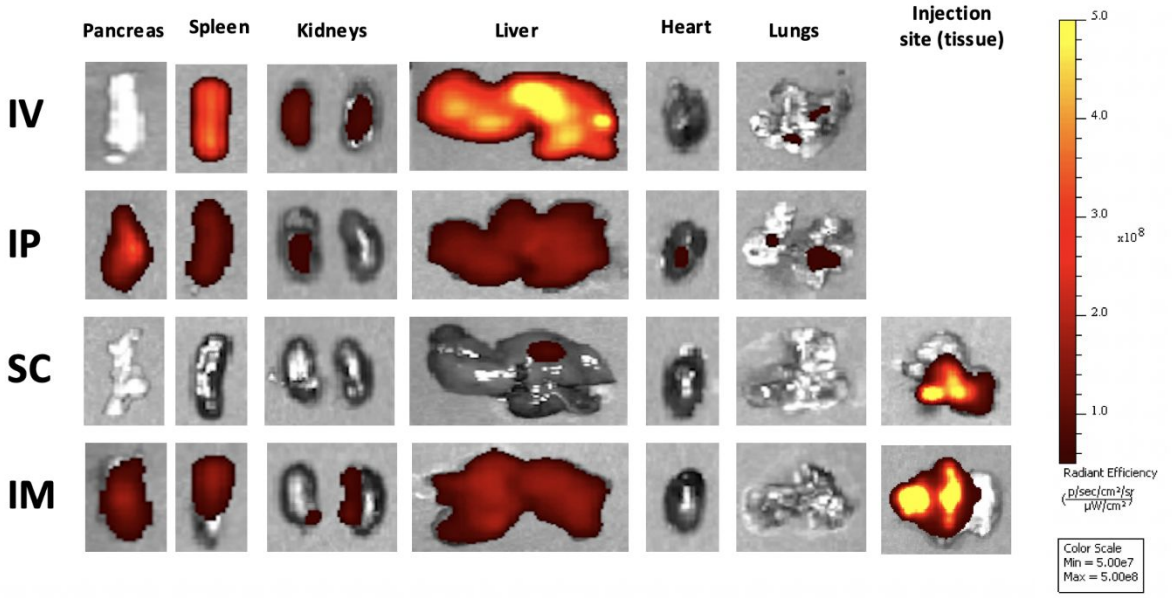


Figure S3. Delivery of $306O_{i10}$ LNPs by different routes resulted in varying biodistribution profiles. Biodistribution was determined 1 hour after injection of $306O_{i10}$ LNPs carrying 0.5 mg/kg Cy5 mRNA by

various routes. Organs were excised and imaged for fluorescence by IVIS. For SC and IM injections, the tissue surrounding the injection site was also excised and imaged. Biodistribution occurred predominantly in the liver and spleen for IV and IP injections. LNPs accumulated almost entirely at the injection site for SC and IM injections, with a lesser amount accumulating in the liver.

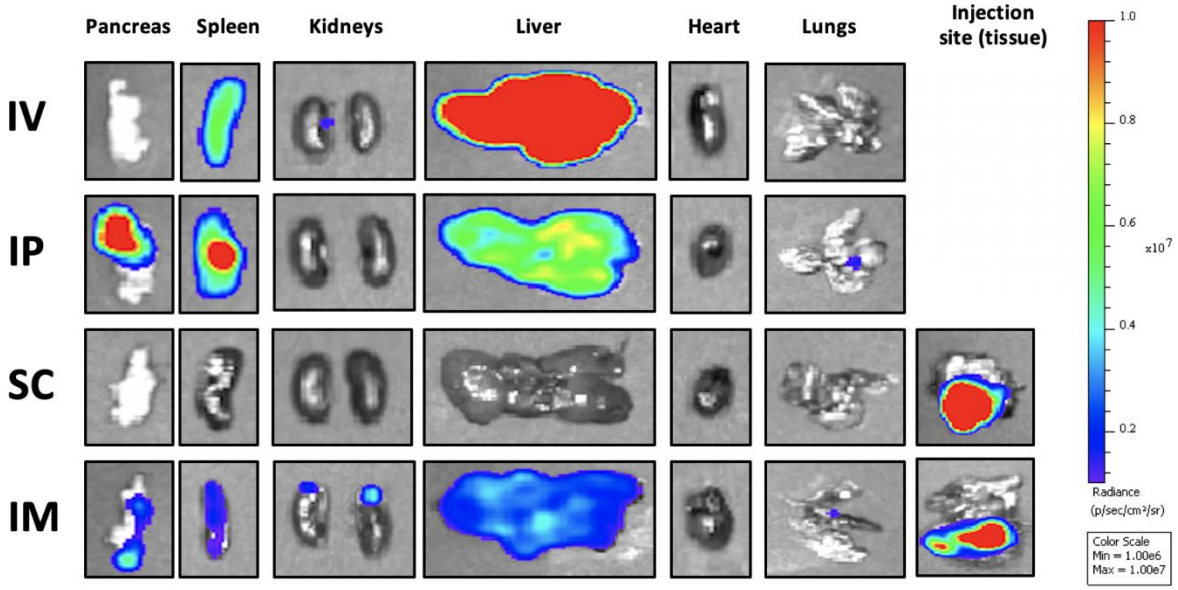


Figure S4. Delivery of $306O_{i10}$ LNPs by different routes resulted in varying protein expression profiles. Bioluminescence was determined 6 hours after injection of $306O_{i10}$ LNPs carrying 0.5 mg/kg Firefly luciferase mRNA by various routes. Organs were excised and imaged for bioluminescence by IVIS. For SC and IM injections, the tissue surrounding the injection site was also excised and imaged. Expression occurred almost entirely in the liver for IV injections, while IP delivery resulted in expression in the liver, spleen, and pancreas. Luciferase was expressed entirely at the injection site for SC injections. IM delivery also resulted in expression at the injection site and to a lesser extent in the liver.

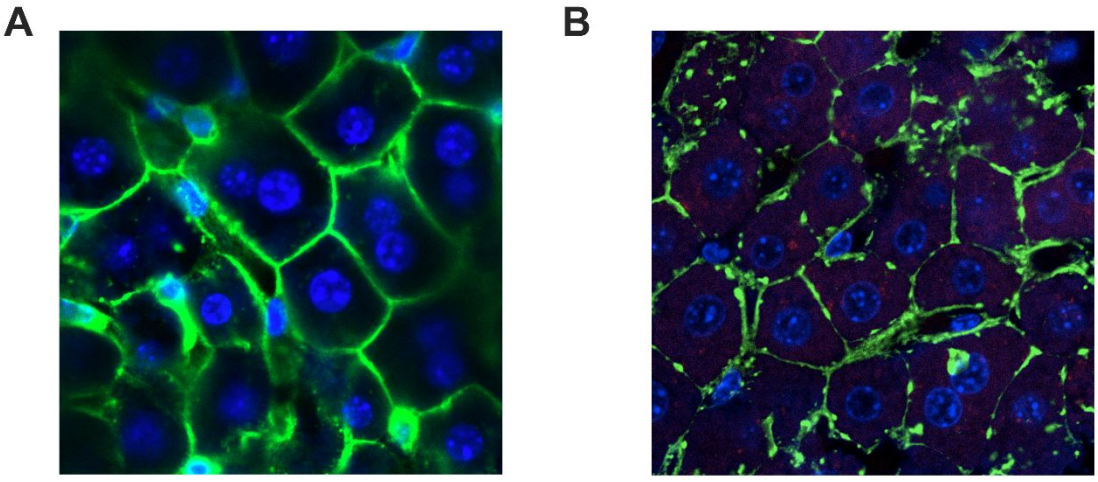


Figure S5. Confocal microscopy confirmed mCherry expression in mouse liver cells. Mice were injected by tail vein with naked mCherry mRNA or $306O_{i10}$ LNPs carrying mCherry mRNA (both at dose of 0.5 mg/kg). Six hours post-injection, mice were sacrificed and the organs were excised, fixed,

sectioned, and stained for confocal microscopy. Tissues were stained with Phalloidin (actin) and Hoechst (cell nuclei). (A) No mCherry expression was observed in mice treated with naked mRNA. (B) mCherry expression (red signal) is observed in the cells of mice treated with LNPs carrying mRNA.