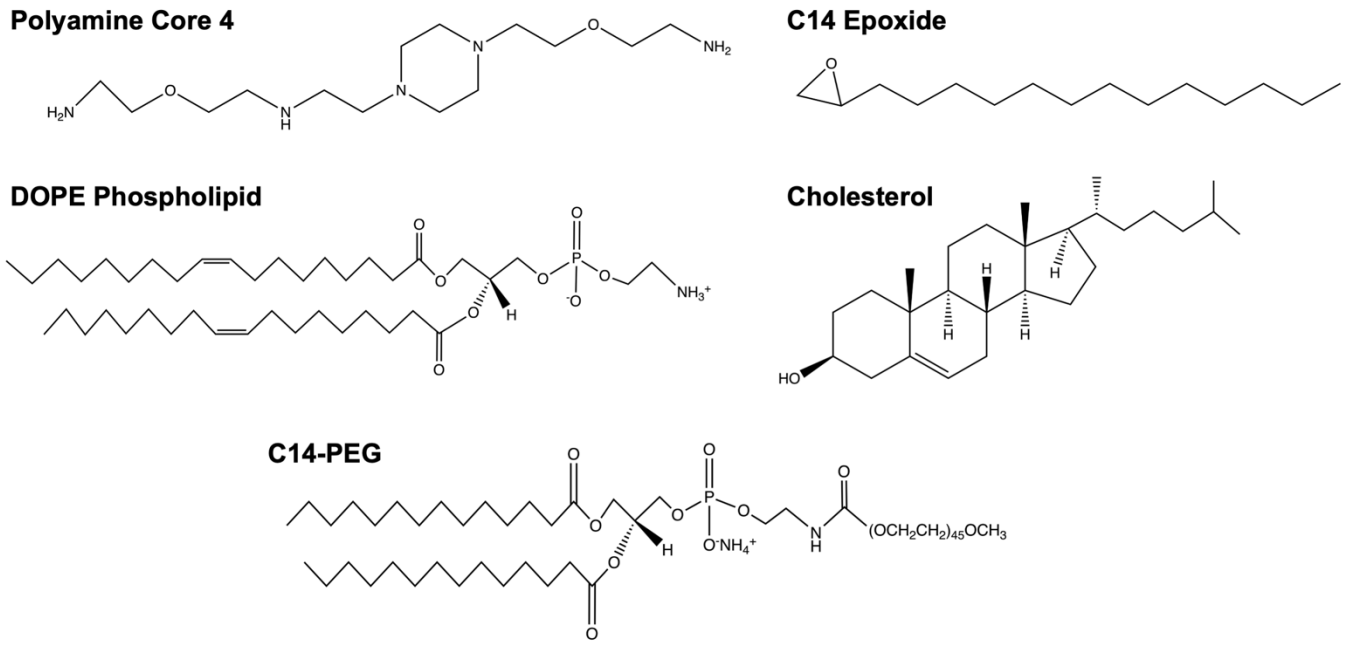
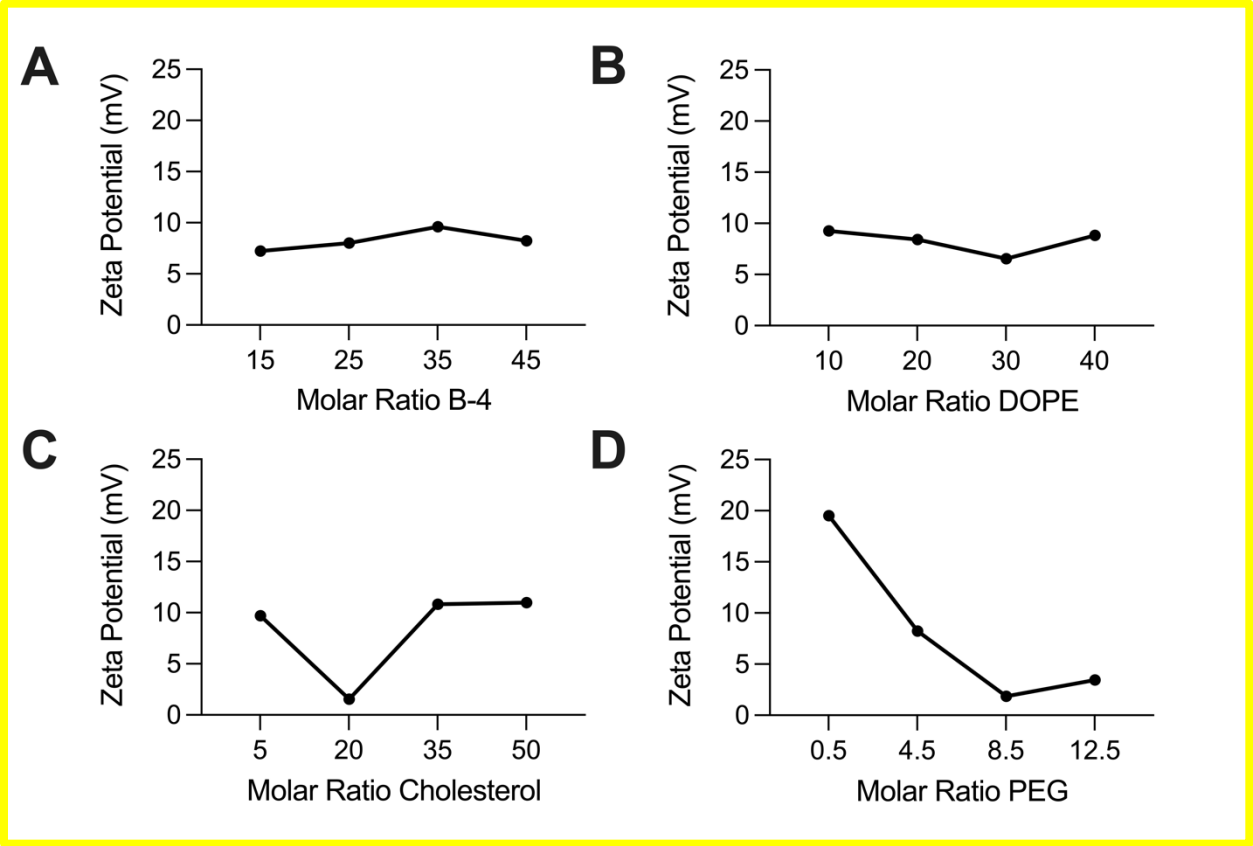
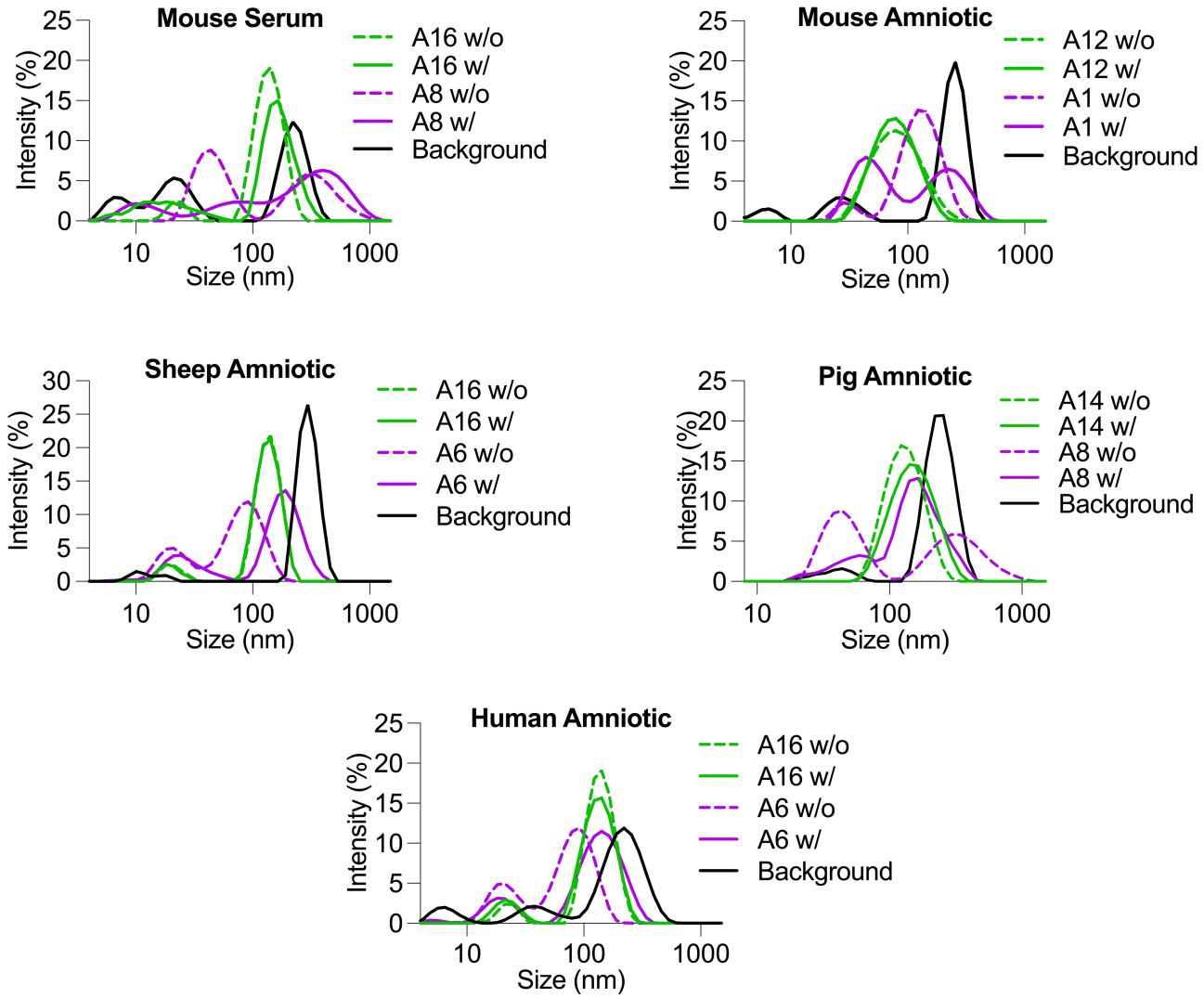
8. Supplementary Data



Supplementary Figure 1– Chemical structures of the excipients used in LNP formulation. *As detailed in materials and methods, polyamine core 4 and C14 epoxide were reacted to synthesize the ionizable lipid used in LNP formulations. Other LNP excipients include DOPE (phospholipid), cholesterol, and the C14-PEG conjugate.*



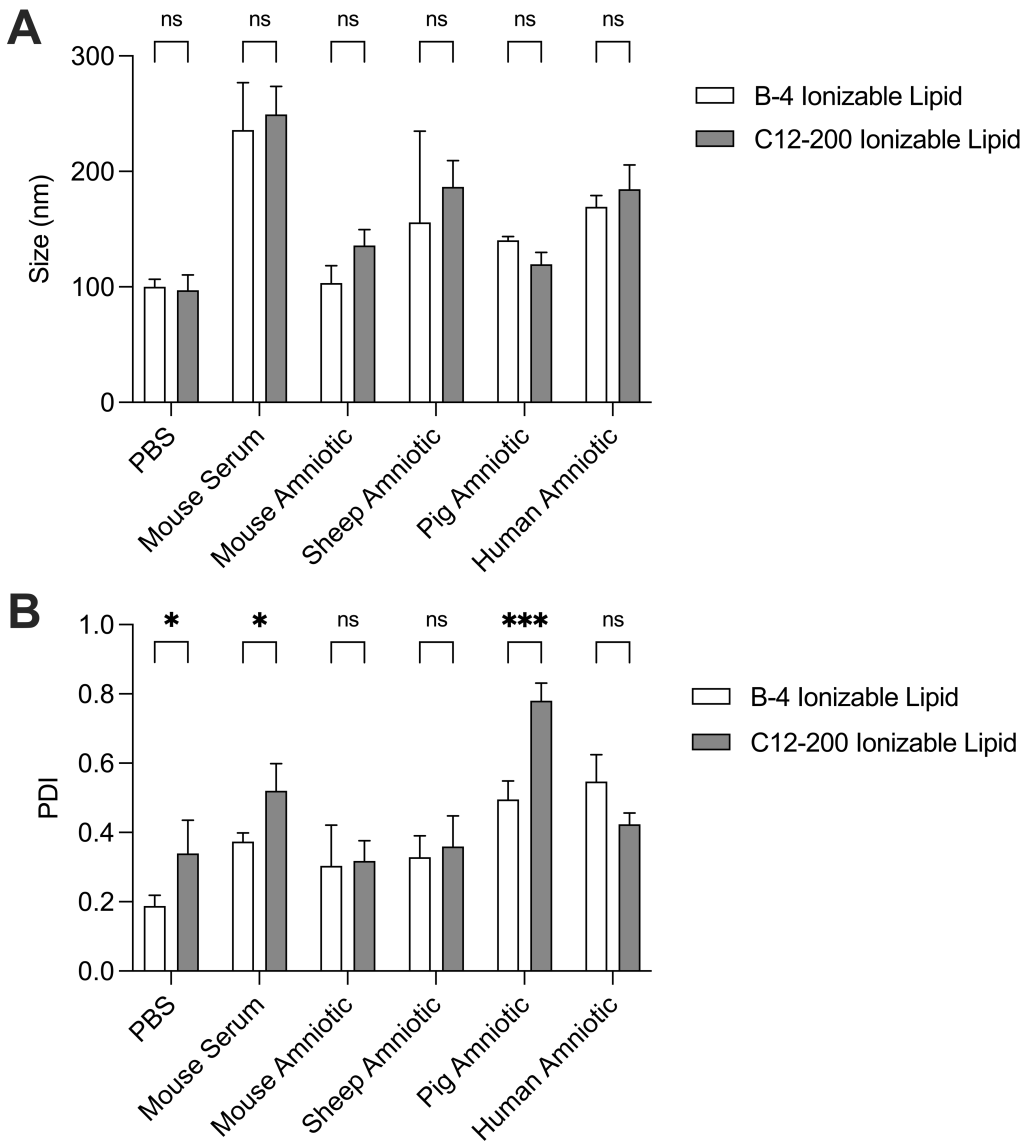
Supplementary Figure 2 – LNP structure function relationships with zeta potential. *Each data point represents an average of the zeta potential for each of the four LNPs with the given excipient molar ratio.* ***(A), (B), (C)*** *No noticeable trend with increasing molar ratios of ionizable lipid B-4, DOPE, and cholesterol with zeta potential.* ***(D)*** *Zeta potential decreases as the molar ratio of PEG increases.*



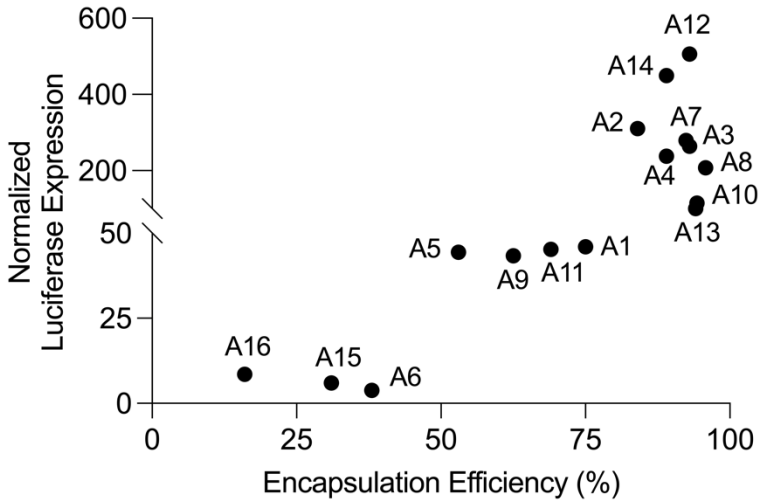
Supplementary Figure 3 – Representative intensity (%) vs. size (nm) curves for most and least stable LNPs in each of the five fluids evaluated*. Most stable particles in PBS alone (dashed) and with each fluid (solid) are shown in green. Least stable particles in PBS alone (dashed) and with each fluid (solid) are shown in purple. The background intensity curve of each fluid is shown in black.*

Supplementary Table 1. *Characterization of mouse serum and fetal fluids used in library screen.*

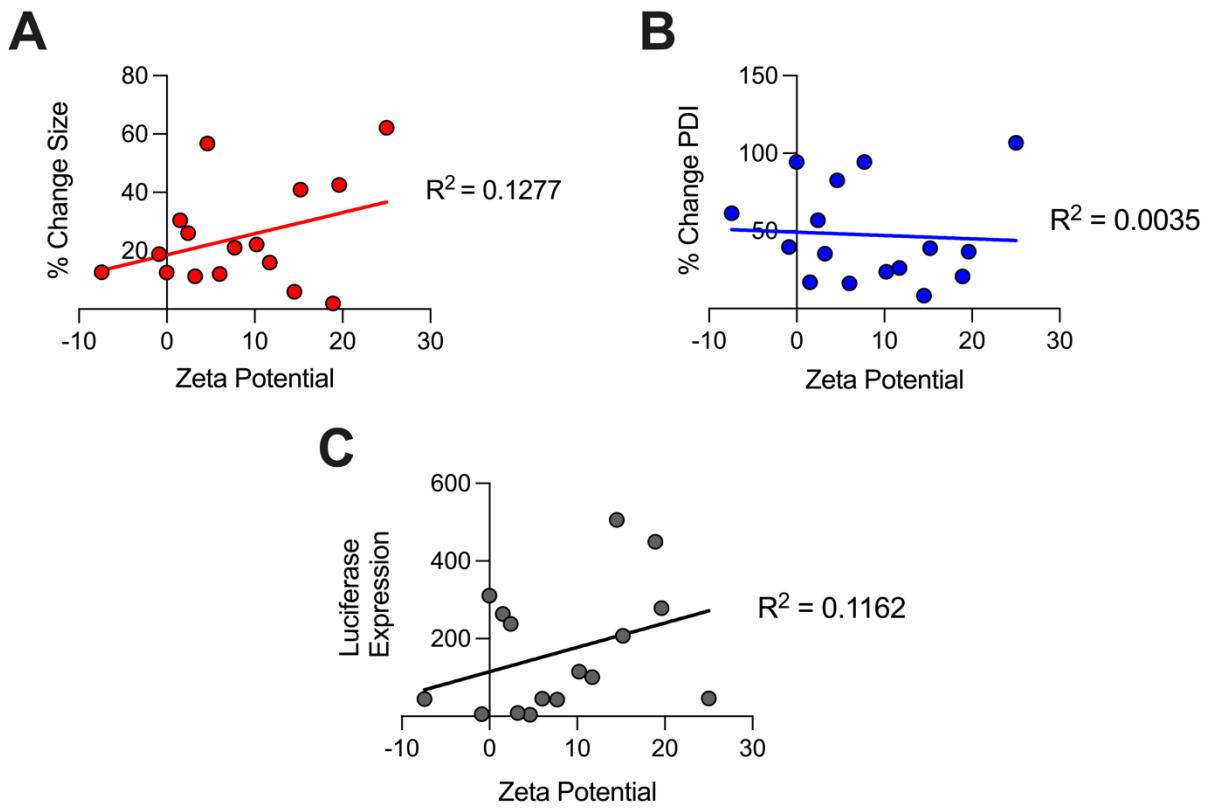
Fluid	pH	Protein Concentration (ng/µL)
Mouse Serum	8.45 ± 0.10	1507 ± 60
Mouse Amniotic	7.58 ± 0.11	144 ± 2
Sheep Amniotic	8.07 ± 0.13	515 ± 12
Pig Amniotic	7.77 ± 0.09	308 ± 9
Human Amniotic	8.1 ± 0.06	140 ± 2



Supplementary Figure 4 - Effect of different ionizable lipids for A5 formulation on ex vivo size and PDI stability measurements. *Two A5 LNPs were formulated with either the B-4 or C12-200 ionizable lipids.* ***(A)*** *No significant difference ($p < 0.05$) in ex vivo size measurements for A5 LNP formulated with B-4 ionizable lipid versus C12-200 ionizable lipid in any of the amniotic fluids tested.* ***(B)*** *A5 LNP formulated with C12-200 ionizable lipid had significantly higher PDI measurements in PBS (*$p < 0.05$), mouse serum (*$p < 0.05$), and pig amniotic (***$p < 0.001$) fluids than the same LNP with B-4 ionizable lipid. These results in fluids are likely explained by the significantly higher PDI of the C12-200 LNP in PBS alone compared to the same formulation with B-4 ionizable lipid.*



Supplementary Figure 5. *Correlation between in vitro LNP-mediated luciferase mRNA delivery and encapsulation efficiency for the LNP library.*



Supplementary Figure 6 – Correlations of stability and in vitro luciferase mRNA delivery with zeta potential for the LNP library in mouse amniotic fluid. *(A) and (B) No correlation between zeta potential and percent change in size or percent change in PDI stability measurements in mouse amniotic fluid for the 16 LNP library. (C) No correlation between zeta potential and in vitro luciferase mRNA delivery for the 16 LNP library in mouse amniotic fluid.*