Improved LNPs for mRNA Delivery to the Pancreas without Cationic Lipids

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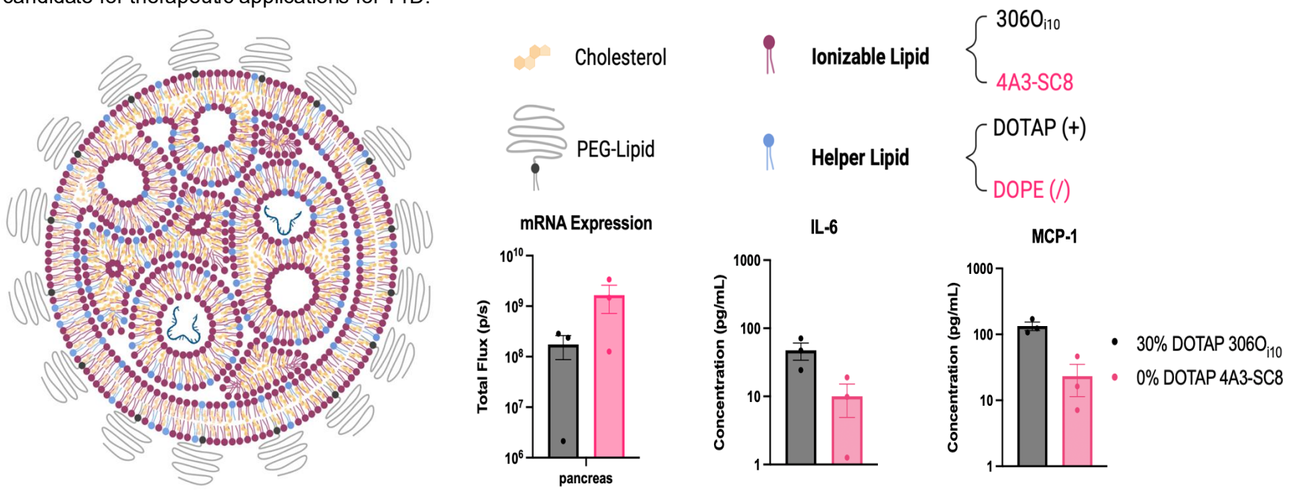
Abstract

Type 1 Diabetes (T1D) is an autoimmune disease that affects about 2 million Americans in which the immune system attacks pancreatic beta-islet cells. This destruction halts insulin production, leading to blood sugar dysregulation. Current treatments involve lifelong insulin therapy, which manages symptoms but does not address the underlying autoimmunity. Monoclonal antibody therapies nonspecifically suppress immune cells, halting autoimmunity, but can leave patients immunocompromised. mRNA-LNPs have the potential to induce tolerizing responses in autoimmune disease models without compromising the immune system. Our group has developed an mRNA-LNP to include positively charged phospholipids (ex, DOTAP) to target the pancreas physicochemically. However, positively charged lipids exacerbate pre-existing inflammation in autoimmune diseases, counterproductive to therapeutic goals. Here, we describe a new mRNA-LNP formulation that does not increase inflammation in non-obese diabetic mice (NOD), a mouse model for T1D, and has a high affinity for the pancreas. We found a DOTAP dose-dependent decrease in IL6 and MCP1 and a 10-fold increase in pancreatic tropism by including the ionizable lipid 4A3-SC8. Its decreased inflammatory profile makes it a strong candidate for therapeutic applications for T1D.



Methods

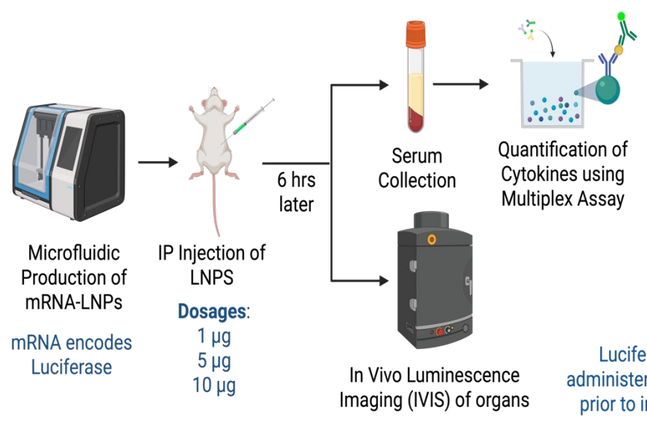


Fig 1. LNPs were produced by microfluidic mixing and characterized by size, charge and mRNA encapsulation efficiency measurements. Next, 5 µg of mRNA-LNPs were injected intraperitoneally to C57Bl/6 or NOD mice. Six hours later, serum was collected and organs were imaged via IVIS. Serum cytokine concentrations were measured using a multiplexed, bead-based assay.

Results

DOTAP Enhances Pancreatic Specificity but Induces Inflammation

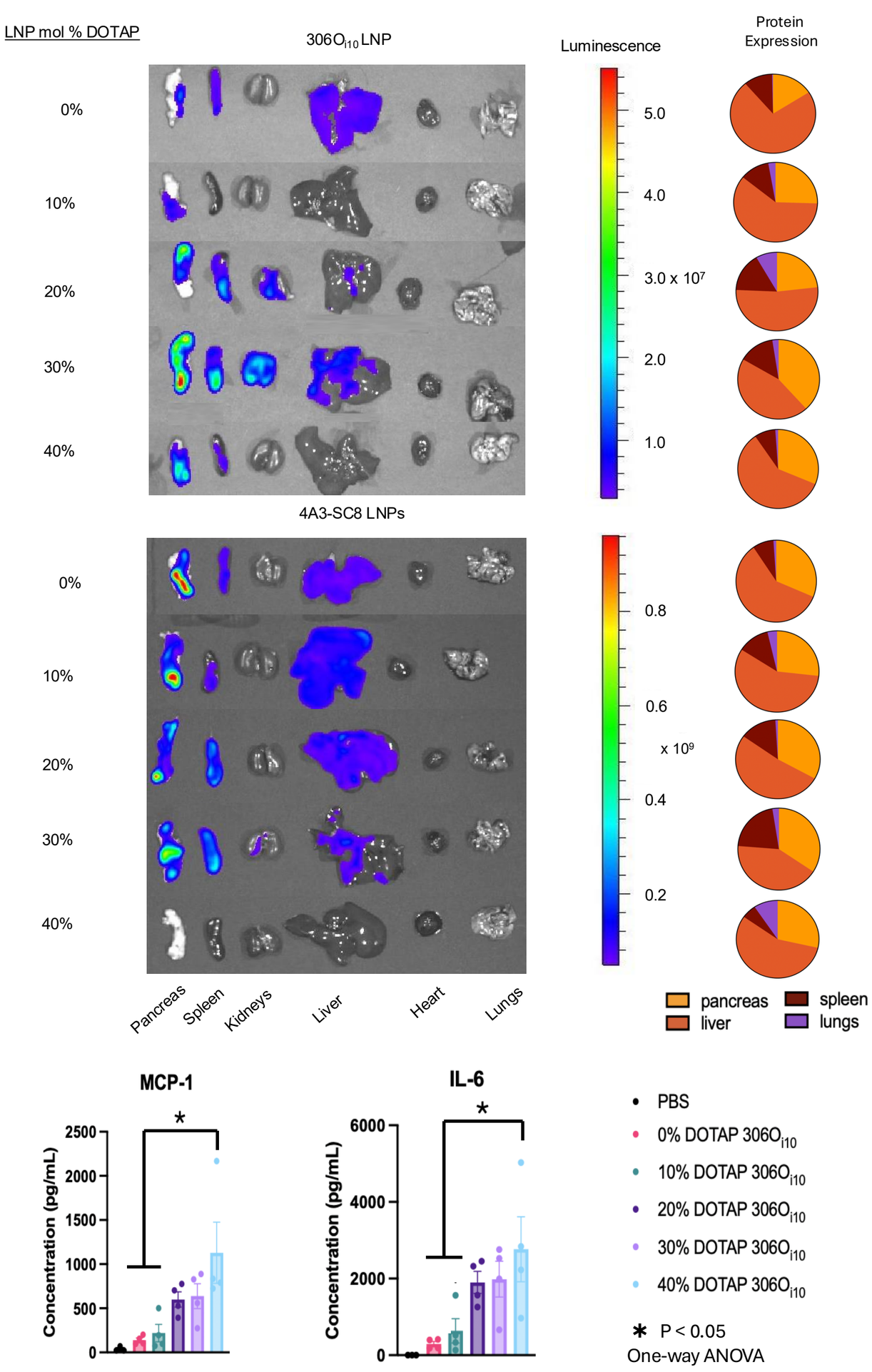


Fig 2. A) By IVIS imaging, DOTAP-containing 306O$_{i10}$ LNPs show enhanced pancreatic specificity, but DOTAP is not necessary in 4A3-SC8 LNPs. **B)** The fraction of luciferase signal detected in each organ measured from the IVIS images in **A**. **C)** LNPs containing higher DOTAP fractions induce greater levels of serum cytokines.

4A3-SC8 LNPs also perform best in NOD Mice

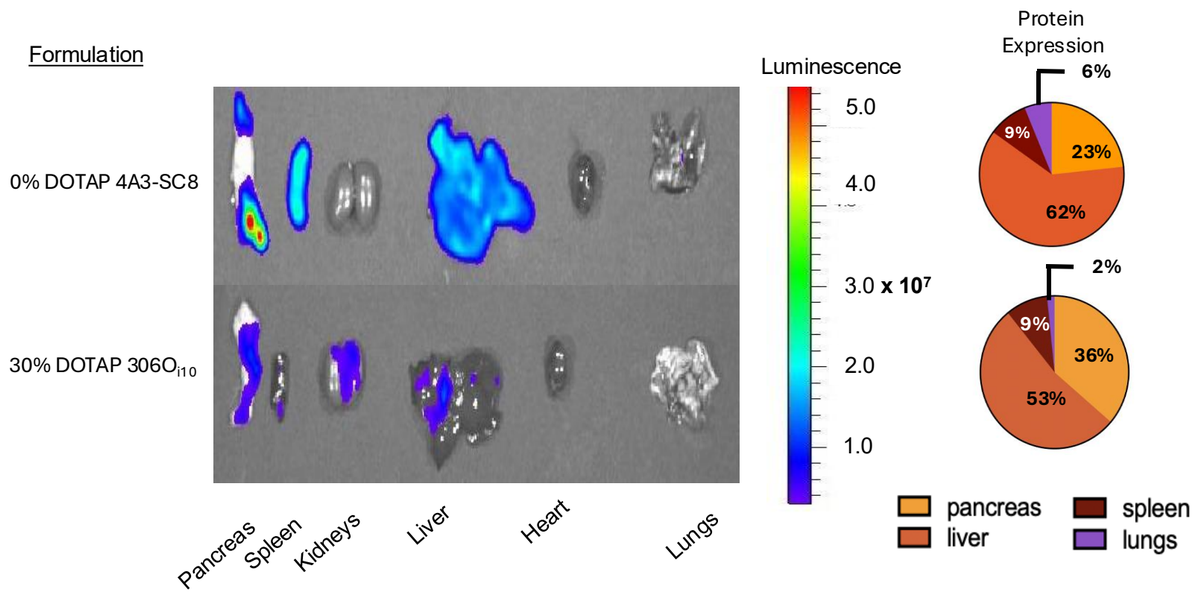


Fig. 3 The top left panel shows IVIS images of NOD mouse organs when treated with top candidates from fig 2. (0% DOTAP 4A3-SC8 & 30% DOTAP 306O$_{i10}$). The top right shows the tissue distribution of the protein expression after treatment of the top candidates. The bottom panel quantifies serum inflammatory cytokines after treatment with the top candidates.

Reduction of Off-Target Expression with mRNA Containing miR-122 Binding Seq

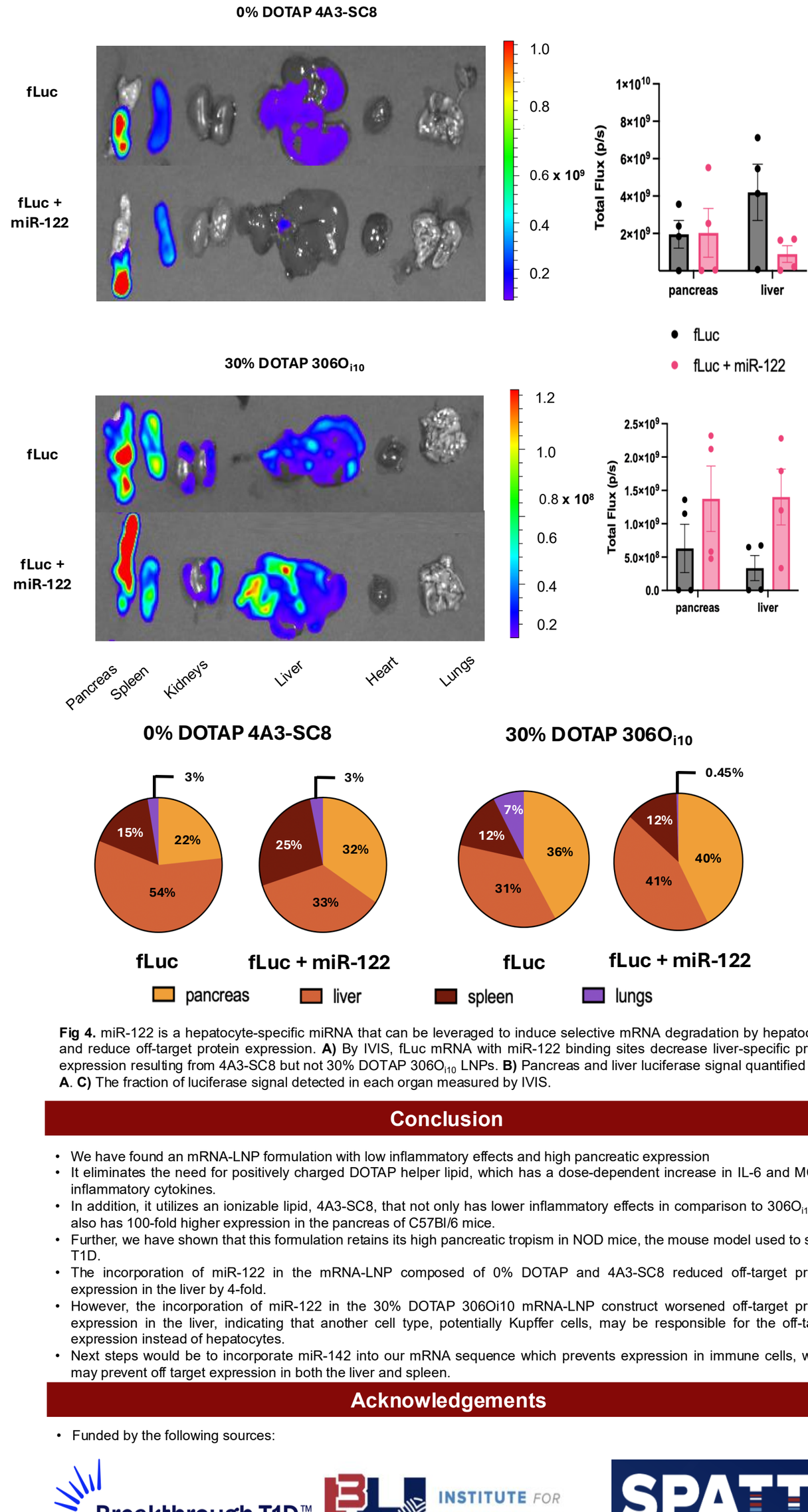


Fig 4. miR-122 is a hepatocyte-specific miRNA that can be leveraged to induce selective mRNA degradation by hepatocytes and reduce off-target protein expression. **A)** By IVIS, fLuc mRNA with miR-122 binding sites decrease liver-specific protein expression resulting from 4A3-SC8 but not 30% DOTAP 306O$_{i10}$ LNPs. **B)** Pancreas and liver luciferase signal quantified from **A**. **C)** The fraction of luciferase signal detected in each organ measured by IVIS.

Conclusion

- We have found an mRNA-LNP formulation with low inflammatory effects and high pancreatic expression
- It eliminates the need for positively charged DOTAP helper lipid, which has a dose-dependent increase in IL-6 and MCP-1 inflammatory cytokines.
- In addition, it utilizes an ionizable lipid, 4A3-SC8, that not only has lower inflammatory effects in comparison to 306O$_{i10}$ but also has 100-fold higher expression in the pancreas of C57Bl/6 mice.
- Further, we have shown that this formulation retains its high pancreatic tropism in NOD mice, the mouse model used to study T1D.
- The incorporation of miR-122 in the mRNA-LNP composed of 0% DOTAP and 4A3-SC8 reduced off-target protein expression in the liver by 4-fold.
- However, the incorporation of miR-122 in the 30% DOTAP 306Oi10 mRNA-LNP construct worsened off-target protein expression in the liver, indicating that another cell type, potentially Kupffer cells, may be responsible for the off-target expression instead of hepatocytes.
- Next steps would be to incorporate miR-142 into our mRNA sequence which prevents expression in immune cells, which may prevent off target expression in both the liver and spleen.

Acknowledgements

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