

Supplemental Information

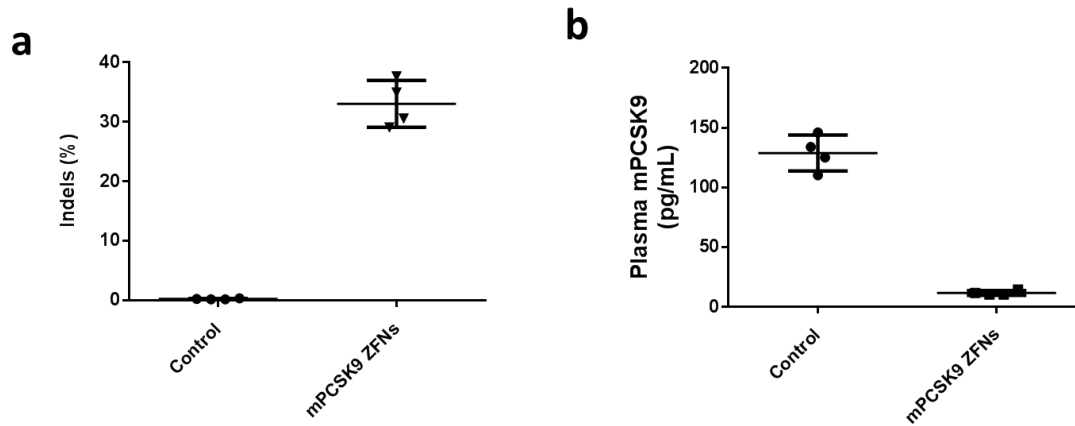
Non-viral Delivery of Zinc Finger Nuclease

mRNA Enables Highly Efficient *In Vivo* Genome

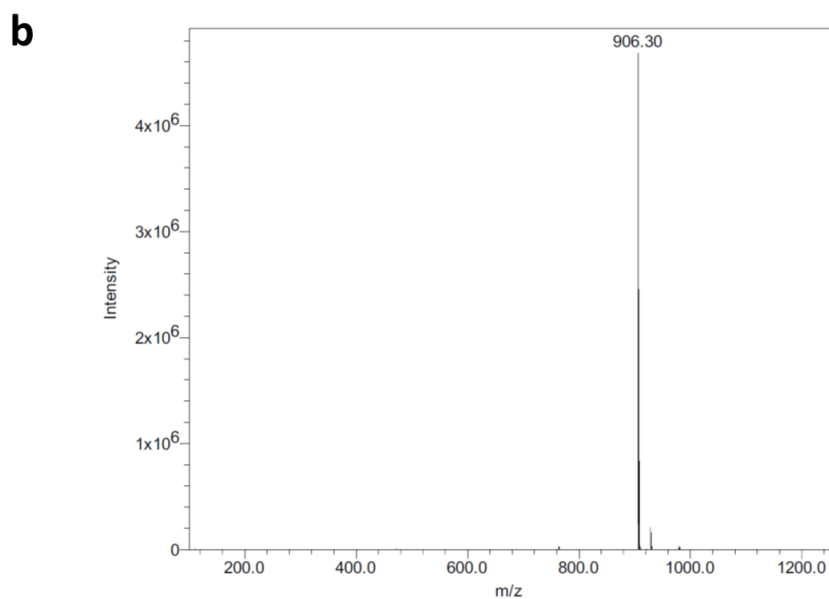
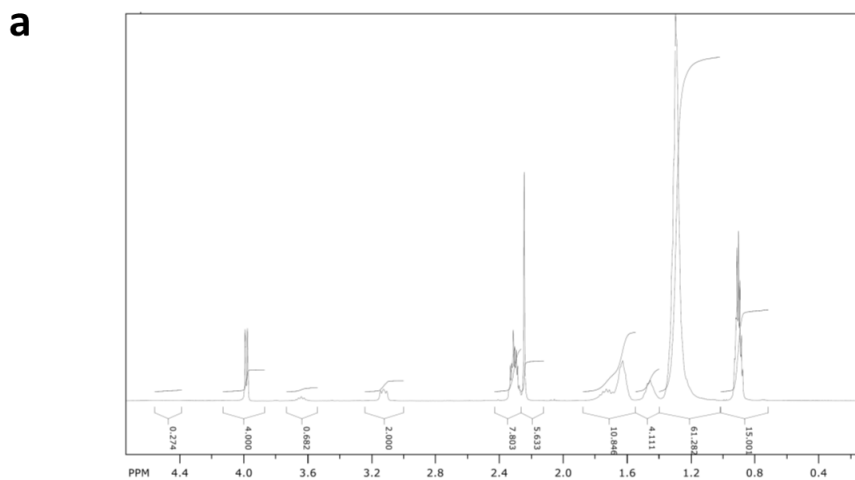
Editing of Multiple Therapeutic Gene Targets

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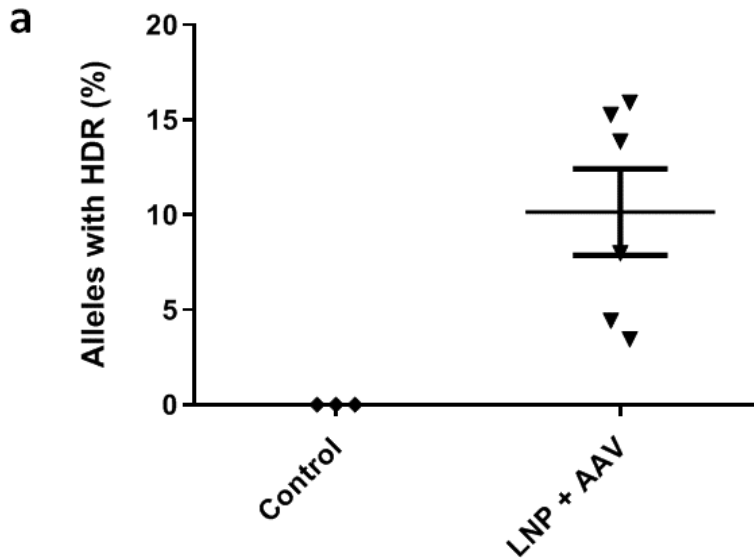
Supplemental Figures



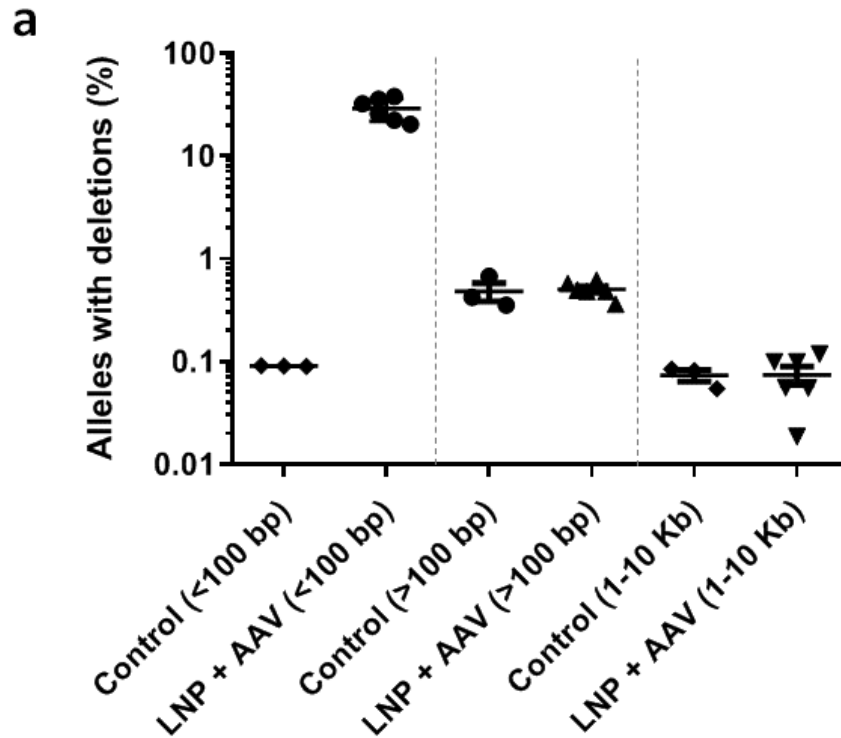
Supplemental Figure 1. Knockout of therapeutically relevant liver gene target *PCSK9*. (a) Liver genome editing results from mice injected with 0.8 mg/kg LNP containing 58780/61748 ZFN mRNAs (targeting murine *PCSK9*). Animals were pre-treated with dexamethasone. ($n = 4$ per group) ($P = 0.00000297$). (b) Liver genome editing results from mice described in Supplemental Figure 1a. ($n = 4$ per group) ($P = 0.0000048$).



Supplemental Figure 2. Characterization of novel ionizable lipid. (a) Proton nuclear magnetic resonance (NMR) spectrum for novel ionizable lipid used in LNP formulations. Transmitter frequency of 400 MHz used. Number of scans = 16. **(b)** Mass spectrum (MS) of novel ionizable lipid used in LNP formulations. Acquired in positive electrospray ionization mode.



Supplemental Figure 3. Genomic quantification of targeted integration efficiency. (a) Fraction of mouse ALB alleles containing a human IDS transgene integrated via the homology directed repair (HDR) pathway within bulk liver genomic DNA harvested from mice injected with a single 0.5 mg/kg administration of LNP containing unmodified 48641/31523 ZFN mRNAs co-injected with 1.5e12 vg/mouse AAV8 hIDS donor (n = 6) compared to control mice injected with buffer alone (n = 3) (P = 0.0188 comparing control to LNP + AAV group).



Supplemental Figure 4. Quantification of large deletions. (a) Fraction of mouse ALB alleles containing small (<100 bp) and large deletions (>100 bp) within bulk liver genomic DNA harvested from mice injected with a single 0.5 mg/kg administration of LNP containing unmodified 48641/31523 ZFN mRNAs co-injected with 1.5×10^{12} vg/mouse AAV8 hIDS donor ($n = 6$) compared to control mice injected with buffer alone ($n = 3$) ($P = 0.812$ comparing control to LNP + AAV group for deletions >100 bp and $P = 0.978$ for 1-10 Kb deletions).