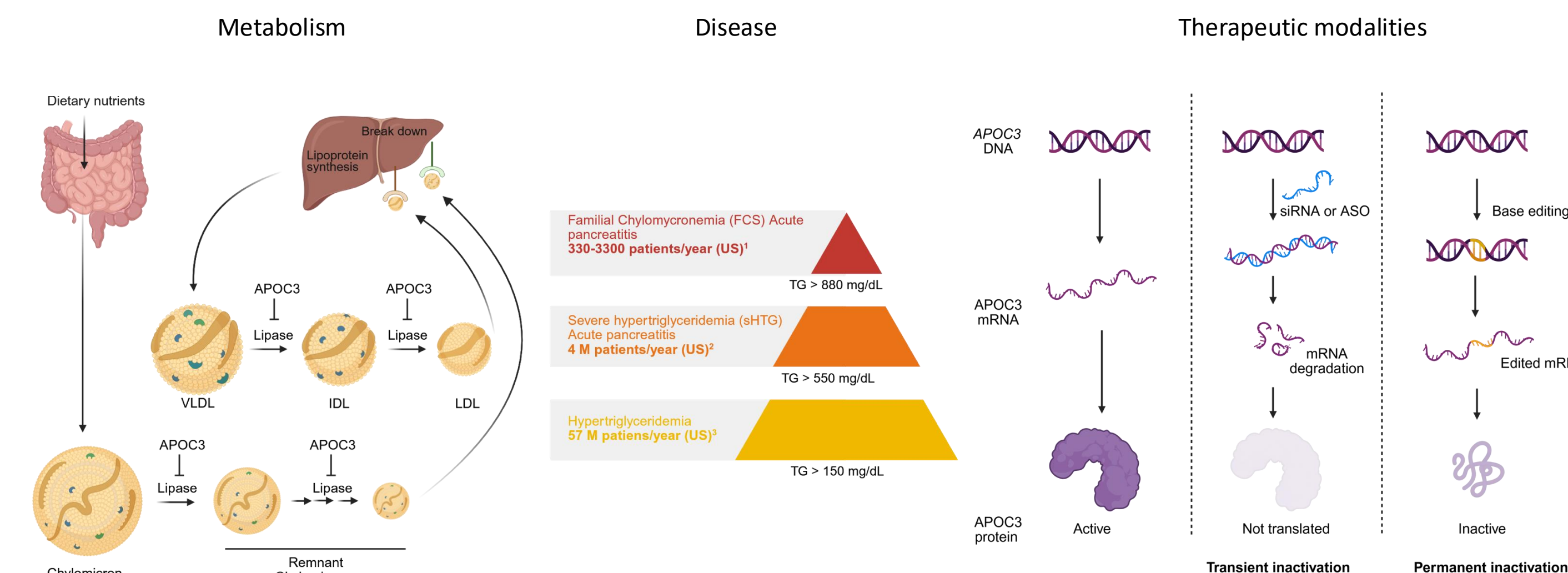


IN VIVO EDITING OF HUMAN APOC3 BY TALE BASE EDITOR NORMALIZES TRIGLYCERIDE LEVELS IN HYPERTRIGLYCERIDEMIC ANIMAL MODEL

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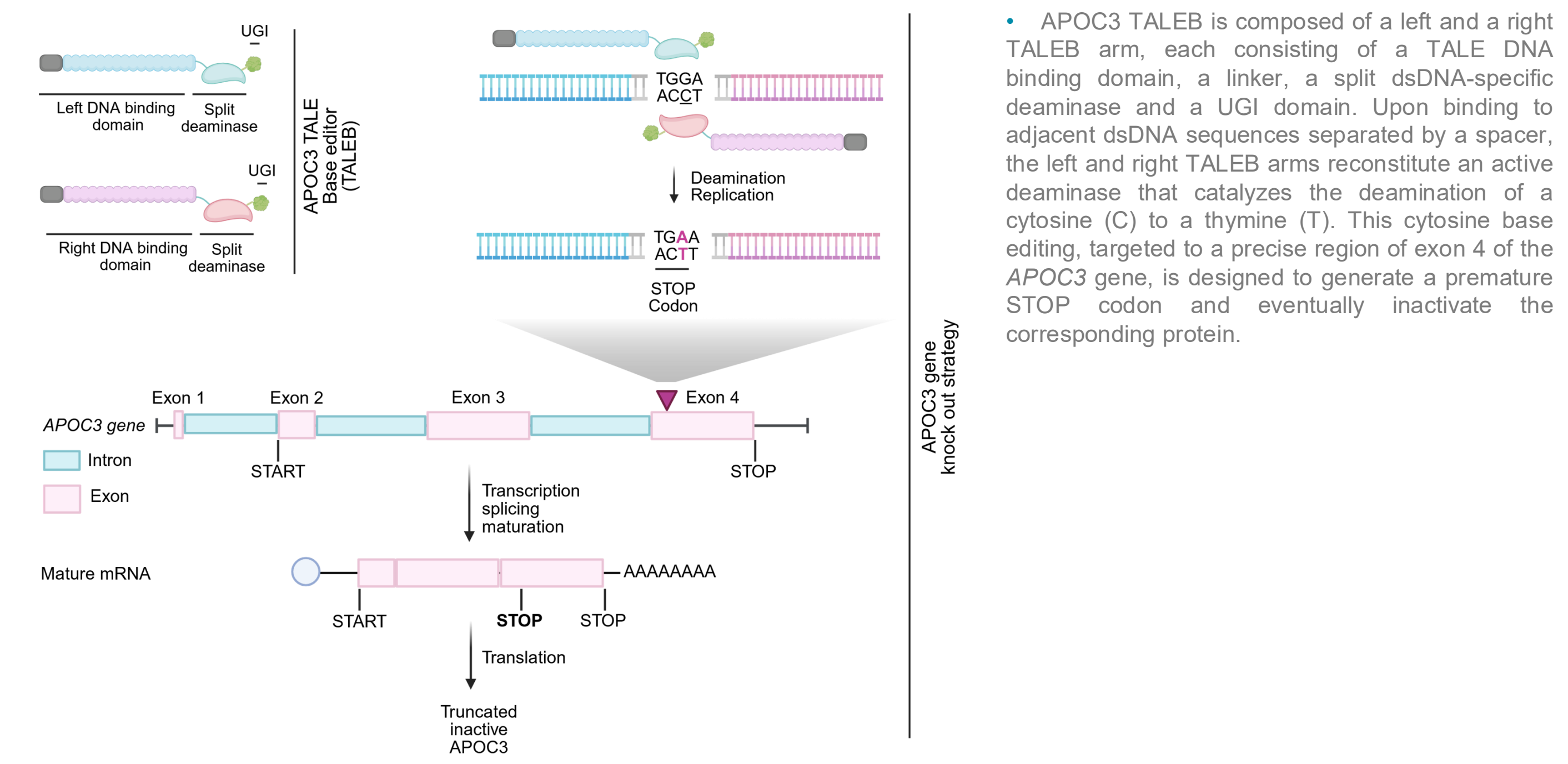
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Background: Hypertriglyceridemia, APOC3 and therapeutic avenues

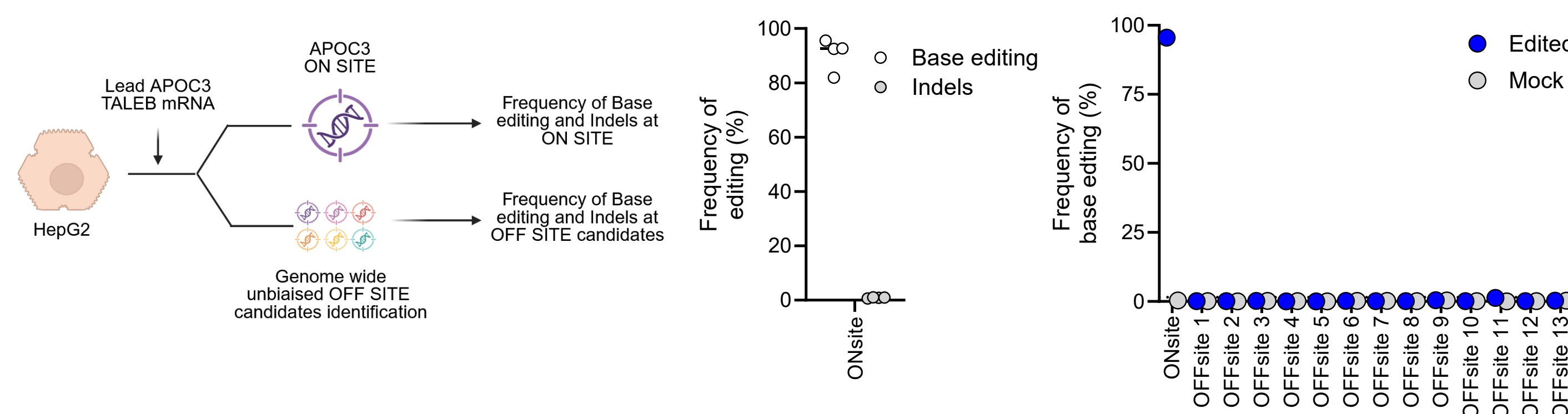
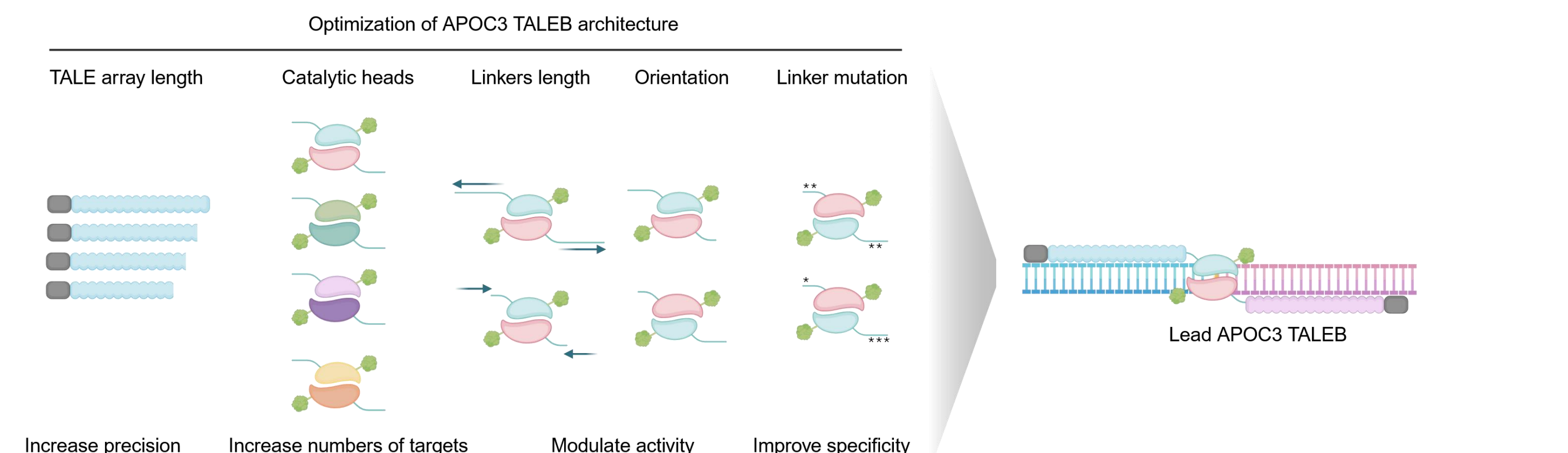


Apolipoprotein C-III (ApoC3), encoded by *APOC3*, is a key regulator of triglyceride-rich lipoprotein metabolism through inhibition of lipoprotein lipase-mediated hydrolysis and hepatic clearance of remnant particles. Excess ApoC3 contributes to severe hypertriglyceridemia (sHTG) and familial chylomicronemia syndrome (FCS) in which impaired clearance of chylomicrons and very low-density lipoprotein remnants drives extreme triglyceride accumulation. Human genetics strongly supports *APOC3* as a therapeutic target, since carriers of naturally occurring loss-of-function variants show reduced triglyceride levels and lower cardiometabolic risk. Several *APOC3*-targeting approaches, including antisense oligonucleotides (ASO) and siRNAs, have demonstrated triglyceride lowering but require repeated administration to maintain effect. Here we report the development of an *APOC3*-specific TALE base editor (TALEB) designed to achieve permanent gene inactivation in vivo using a liver-directed lipid nanoparticle (LNP)-mRNA delivery strategy.

I-Gene editing strategy: Permanent inactivation of APOC3 using TALEB

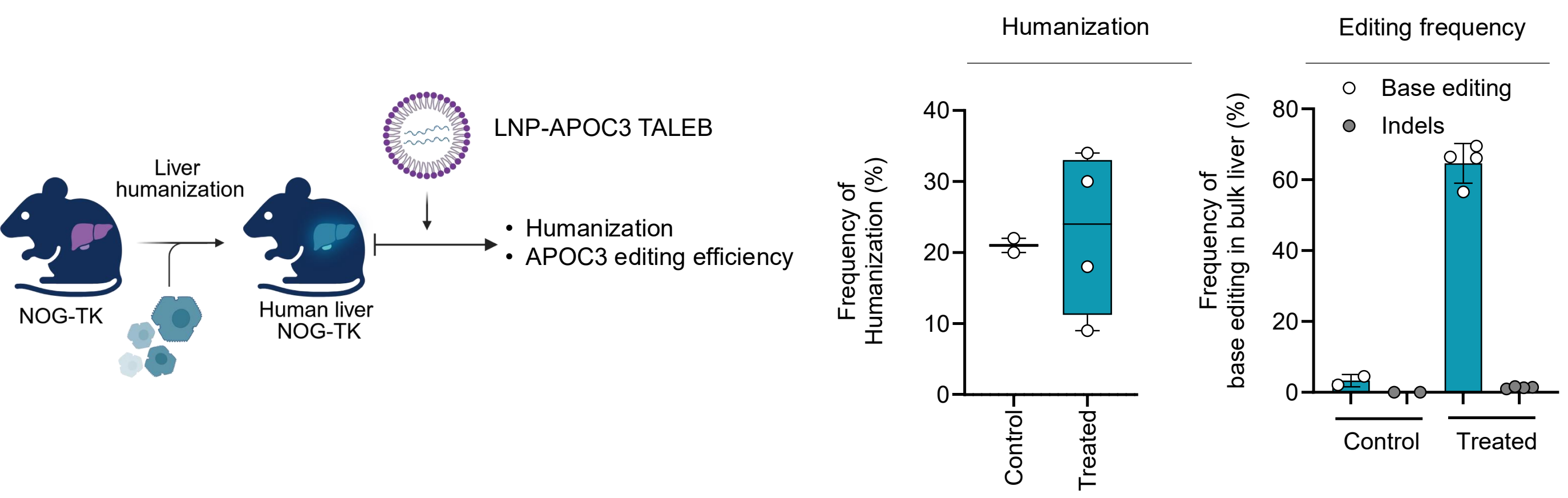


II-Development of the therapeutic APOC3 TALEB: A comprehensive engineering approach



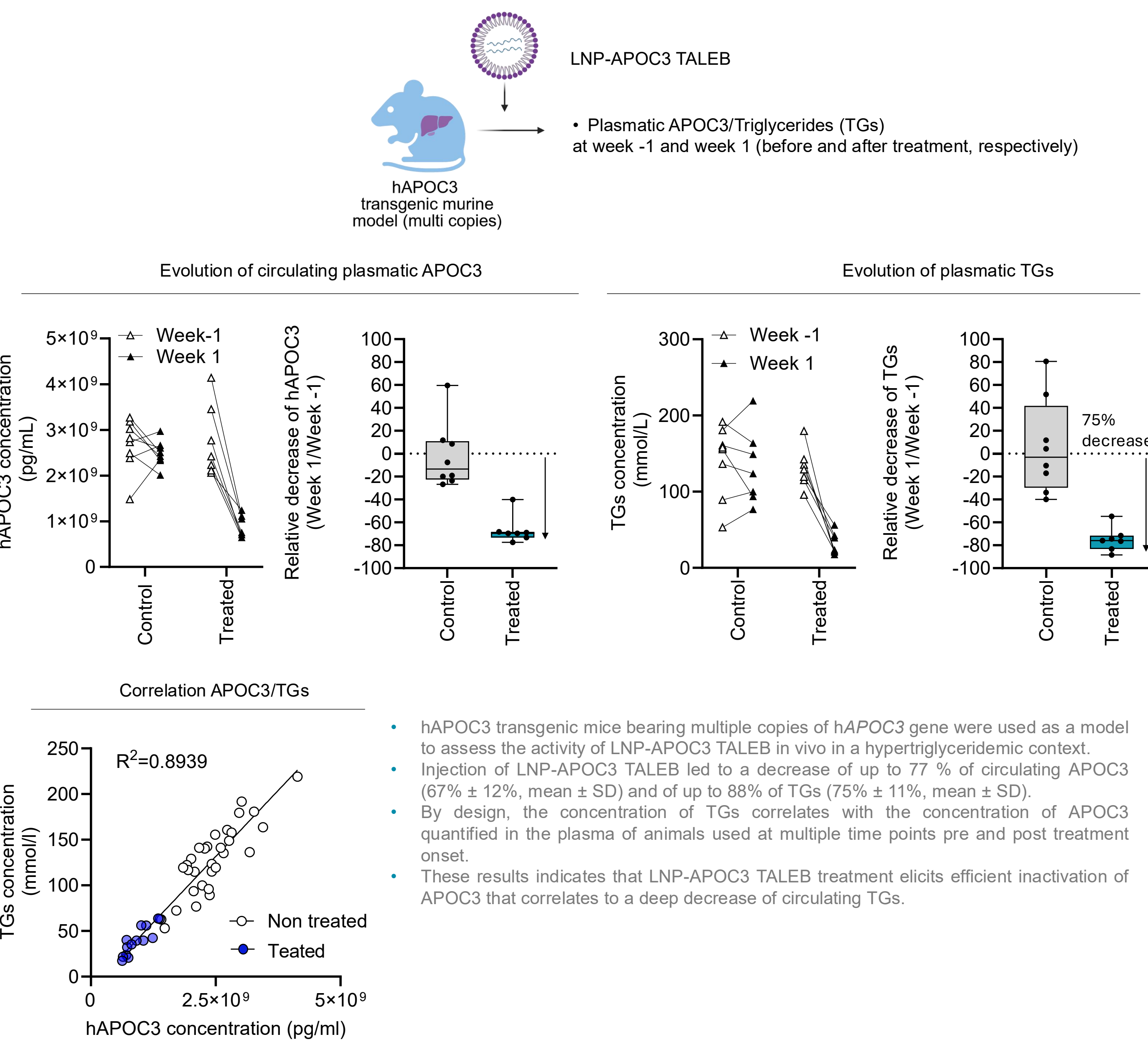
- A comprehensive approach was used to develop the therapeutic lead APOC3 TALEB. Different parameters were evaluated to precisely position the TALEB at the intended target site and optimize its base editing activity and specificity. Several constructs were screened to identify the final lead APOC3 TALEB candidate.
- The activity of the lead APOC3 TALEB was evaluated in vitro using HepG2 cell lines. mRNA encoding the left and right arms of APOC3 TALEB were electroporated in HepG2 and the genomic DNA of treated cells was analyzed by high throughput DNA sequencing of ON site-specific PCR amplicon. Results showed a mean frequency of APOC3 base editing of $\sim 90.7\% \pm 6.0\%$ and $0.9\% \pm 1.6\%$ of insertion and deletion (Indel) events (mean $\pm$ SD).
- The specificity of the lead APOC3 TALEB was evaluated in vitro in HepG2 cell lines using a genome wide and unbiased OFF site identification method. This procedure allowed to identify 13 OFF site candidates. The frequency of base editing generated at these OFF sites was then quantified by high throughput DNA sequencing of OFF site-specific PCR amplicons. Results confirmed ON site activity without showing significant OFF site activity above the threshold set by the values obtained from Mock transfected HepG2 cells. It indicates that the lead APOC3 TALEB is highly active and specific toward *APOC3* target.

III-Activity assessment of LNP APOC3 TALEB mRNA in vivo: A Humanized-liver murine model to assess APOC3 TALEB editing activity in a relevant human genomic context

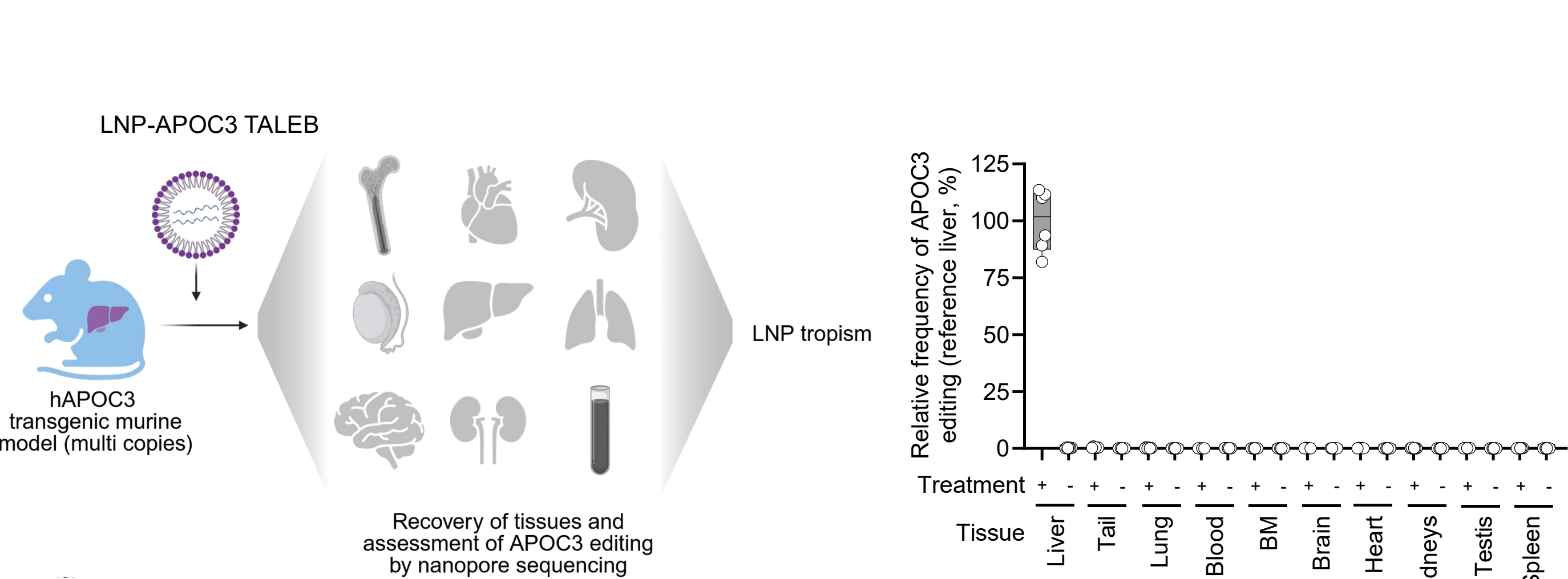


- NOG TK mice were humanized with human primary hepatocytes to create a murine model bearing a human/mouse chimeric liver that was used to assess the activity of LNP-APOC3 TALEB in vivo.
- Liver humanization rate ranged from 10 to 35 %
- Injection of LNP-APOC3 TALEB led to up to 70 % editing of hAPOC3 ($64.6\% \pm 5.5\%$ mean $\pm$ SD) and negligible Indel events ($1.3\% \pm 0.3\%$ mean $\pm$ SD) in the bulk liver cell population. Since hepatocytes represent about 70% of the total liver cell population, these results suggest that most hepatocytes were edited by LNP-APOC3 TALEB.

IV-Activity assessment of LNP APOC3 TALEB mRNA in vivo: Hypertriglyceridemic human APOC3 transgenic murine model



V-Tropism of LNP APOC3 TALEB mRNA in vivo: Hypertriglyceridemic human APOC3 transgenic murine model



- hAPOC3 transgenic mice bearing multiple copies of hAPOC3 gene were used as a model to assess the tropism of LNP-APOC3 TALEB in vivo in a hypertriglyceridemic context.
- Using hAPOC3 on site editing as a read out of tropism, our results show that LNP-APOC3 TALEB is only active in the liver without significant activity in other relevant tissues compared to control (bone marrow, testis, brain, heart, kidney, spleen, lung and whole blood). Relative frequency of APOC3 editing obtained after LNP-APOC3 TALEB treatment (+) or after PBS treatment (-) are illustrated for all tissues.
- This results indicates that the LNP-APOC3 TALEB specifically targets the liver in this hypertriglyceridemic murine model.

Conclusions: Liver-directed in vivo editing of APOC3 by TALE base editing transforms a genetically validated target into a potentially one-time treatment for hypertriglyceridemia.

- A comprehensive engineering strategy enabled the development of a highly active and specific APOC3 TALEB candidate exhibiting robust on-target editing and minimal indel formation.
- In vivo delivery of APOC3 TALEB mRNA formulated in liver-targeted LNP achieved efficient editing of human APOC3 in both humanized liver and hypertriglyceridemic transgenic mouse models.
- APOC3 editing translated into substantial reductions in circulating ApoC3 and TGs, with the magnitude of lipid lowering linked to the editing frequency, confirming the intended mechanism of action.
- LNP APOC3 TALEB activity was restricted primarily to the liver, supporting a favorable liver-targeting profile.
- This proof of concept establishes APOC3 TALEB as a promising one-time in vivo gene editing approach for the treatment of hypertriglyceridemia and related APOC3-driven disorders.

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