Axiomer™ mediated RNA editing of PNPLA3 I148M to address hepatic steatosis in MASH

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Introduction

PNPLA3 I148M a variant commonly reported in the MASH population worldwide

Metabolic dysfunction–associated steatohepatitis (MASH) is a prevalent disease with limited treatment options, making it the second leading cause of liver transplantation in the US.[1]

Patatin-like phospholipase domain-containing protein 3 (PNPLA3), a lipase expressed in hepatocytes and liver stellate cells, plays a pivotal role in lipid homeostasis and metabolism. The PNPLA3 I148M variant, found in 20–60% of MASH patients, is the strongest known genetic risk factor for liver disease progression. It disrupts lipid metabolism, causing hepatic lipid droplet (LD) buildup (steatosis), cellular stress, liver fibrosis, and disease progression to liver cirrhosis.[2,3]

A therapeutic approach addressing this genetic risk factor could alleviate hepatic steatosis and prevents the progression to MASH.

Axiomer™ editing oligonucleotides (EONs) as a potential therapeutic approach

Adenosine deaminase acting on RNA (ADAR) are RNA editing enzymes naturally present in all human cells. They acts on double stranded RNAs and specifically edit A-to-I, changing Adenosine (A) to Inosine (I). During translation Inosine is read as a G, enabling the possibility to change a codon and consequentially the protein sequence. By learning from this natural process, we have developed EONs which consist of short single stranded RNA molecules that are complementary to a target RNA. By binding to RNA, EONs create a double stranded structure which will attracts ADARs and allow the specific A-to-I edit to be performed (**Fig. 1**).

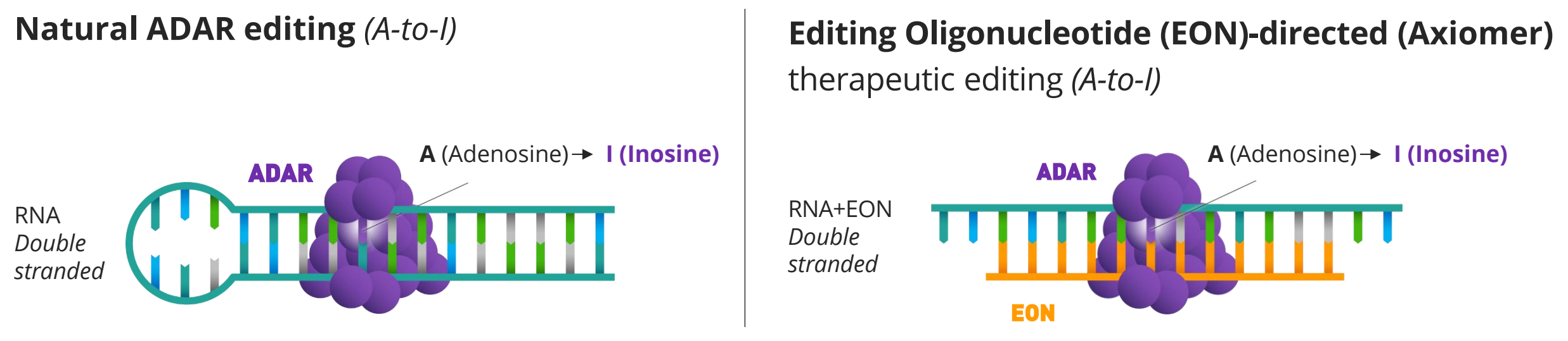


Figure 1. Schematic representation of A-to-I natural ADAR editing (*On the left)*, EON-directed therapeutic editing.

Objectives

- **To investigate how Axiomer™ technology can become a potential therapeutic approach for MASH**
- **To identify the most efficient EONs targeting *PNPLA3* I148M in primary human hepatocytes (PHHs) *in vitro***
- **To assess the effect of EONs on liver steatosis as a result of *PNPLA3* M148V correction in a mouse disease model compared vs. *PNPLA3* I148M Knockdown (KD) approach**

Results

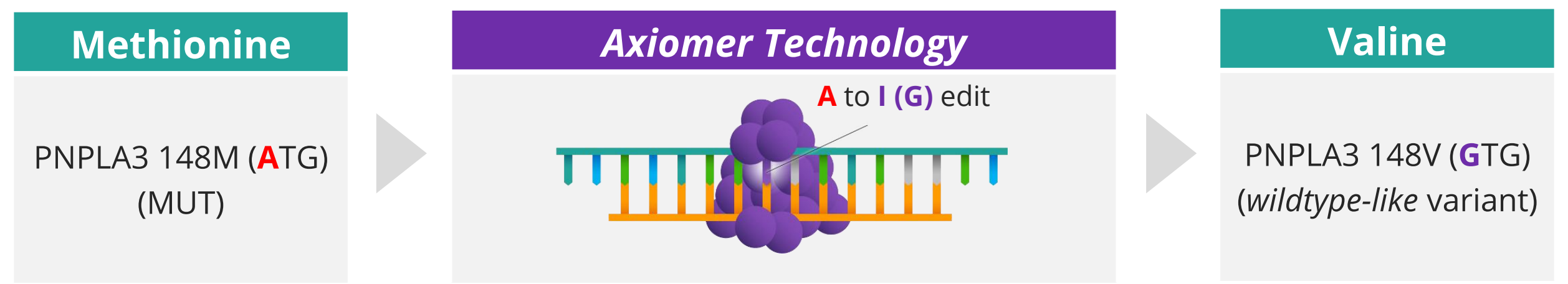
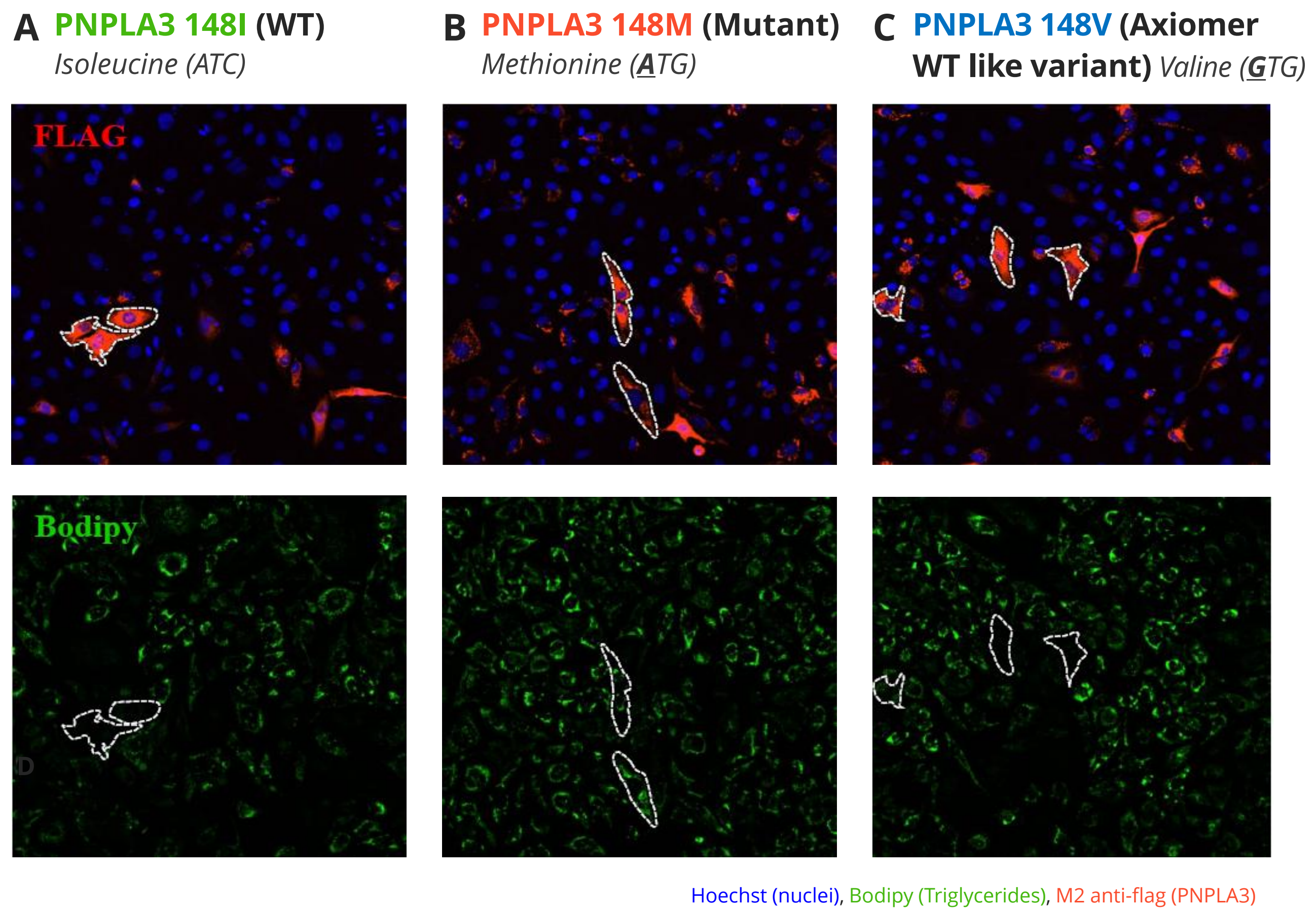


Figure 2. Schematic representation of PNPLA3 148M mutant sequence edited via ADAR leading to PNPLA3 148V wildtype-like variant.

Axiomer™ converts PNPLA3 148M to 148V resembling wild type 148I

Axiomer™ mediated RNA editing can be used to convert PNPLA3 148M mutant to a *de novo* 148V variant (**Fig.2**). To investigate if PNPLA3 148V variant can function as WT, PNPLA3 148V (Axiomer variant) (**Fig. 3C**), 148M (mutant) (**Fig. 3B**) and 148I (WT) (**Fig. 3A**) variants were expressed in HeLa cells loaded with 250μM of Linoleic acid for 24h. Confocal imaging revealed that PNPLA3 148V (Bodipy, mean area per cell) showed similar levels of triglycerides and lipid droplet sizes compared to PNPLA3 WT versus PNPLA3 148M. (**Fig. 3D**). This data shows that PNPLA3 148V lipid homeostasis regulation resembles that of the WT 148I variant



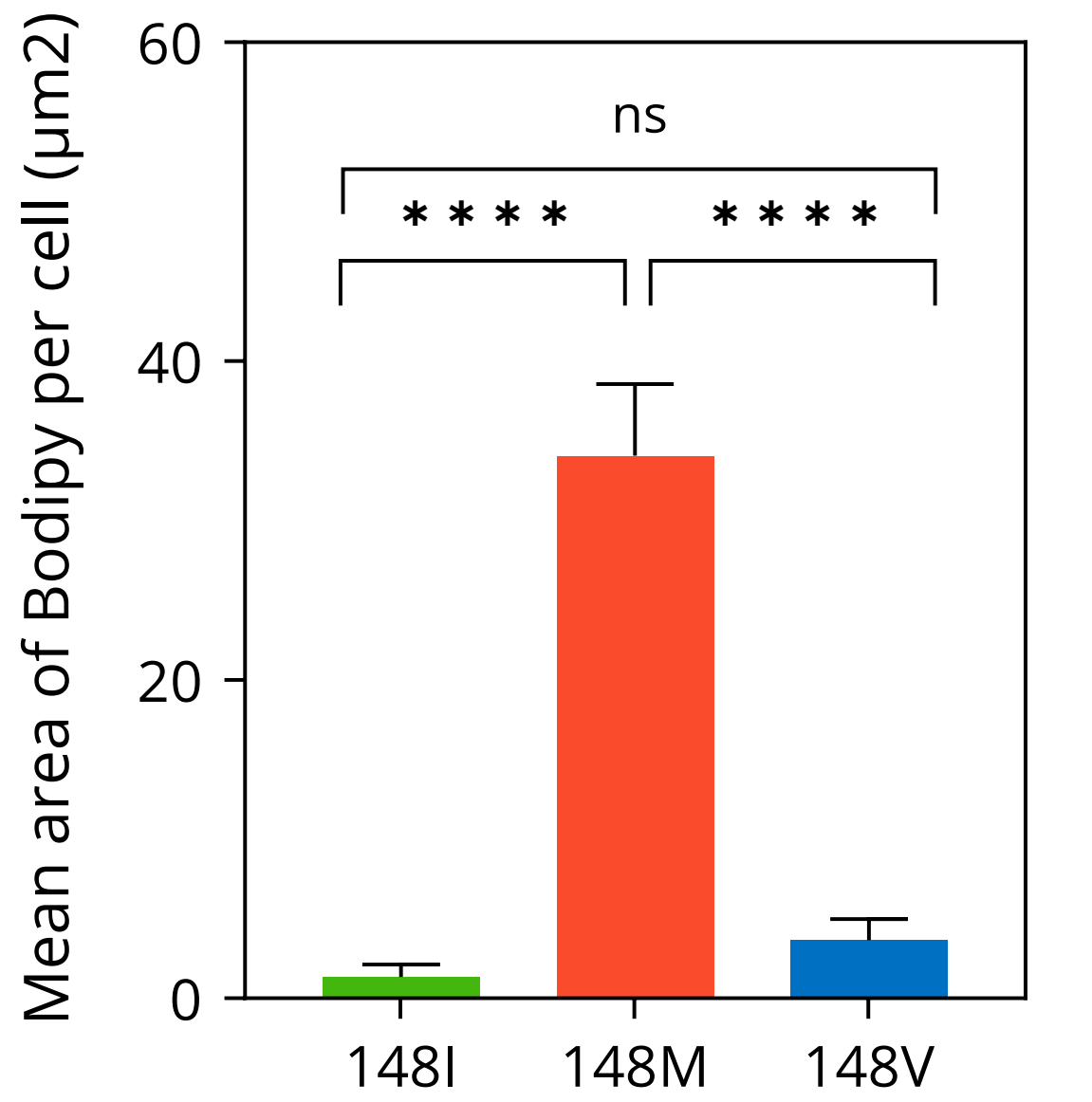


Figure 3. Fluorescent staining (Bodipy; green) of TG in HeLa cells transfected with different PNPLA3 (red) variants exposed to Linoleic acid (24h). And total cells staining using DAPI (blue). (**A**, **B**, **C**) Comparative analysis of TG and LD content is visualized in (**D**). Cell lipase activity by IF. One-way ANOVA, ****, P<0.0001; Mean, SEM)

High throughput screening (HTS) identifies highly efficient EONs

Over 900 GalNAc–conjugated EONs were screened in homozygous PNPLA3 I148M primary human hepatocytes (PHHs) via gymnotic uptake. This screen identified candidates achieving up to 60% RNA editing efficiency (**Fig. 4**). In order to prioritize EONs for further evaluation in relevant mouse model (FRG PNPLA3 I148M humanized mice[4]), EONs were assessed **1**) in PNPLA3 I148M expressing PHHs isolated from FRG mice for editing. **2**) assessment of EON stability and EC50. **3**) initial *in vivo* proof-of-concept studies in FRG mice engrafted with PNPLA3 I148M expressing donor PHHs to assess editing efficiency. Following these assessments, EON 5 was selected as best candidate for further evaluation *in vivo*.

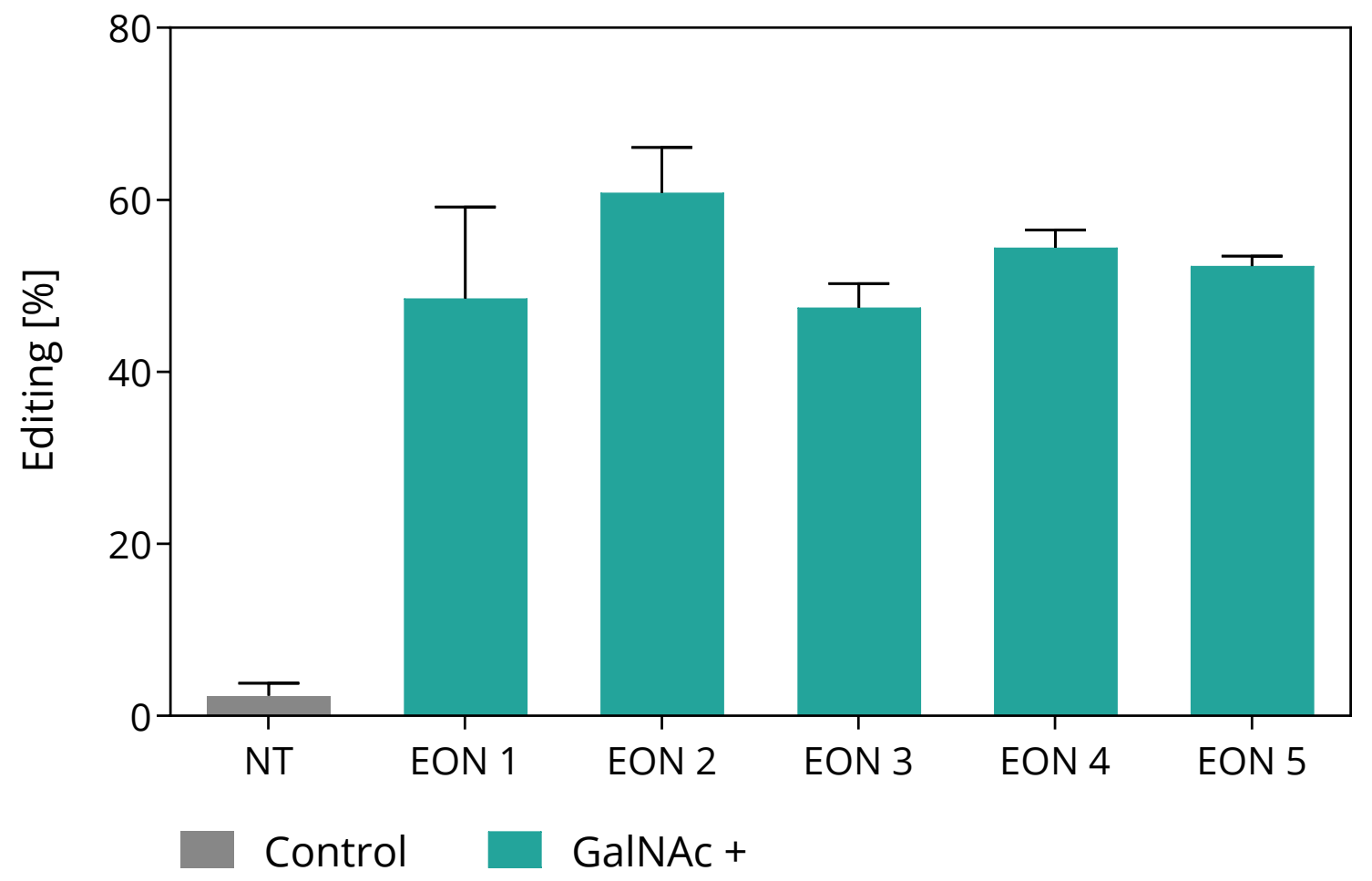
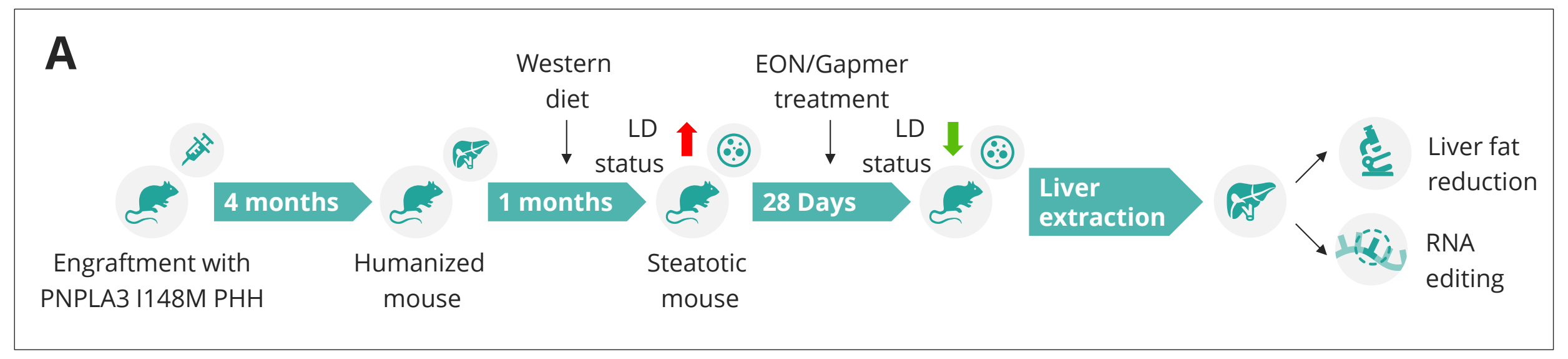


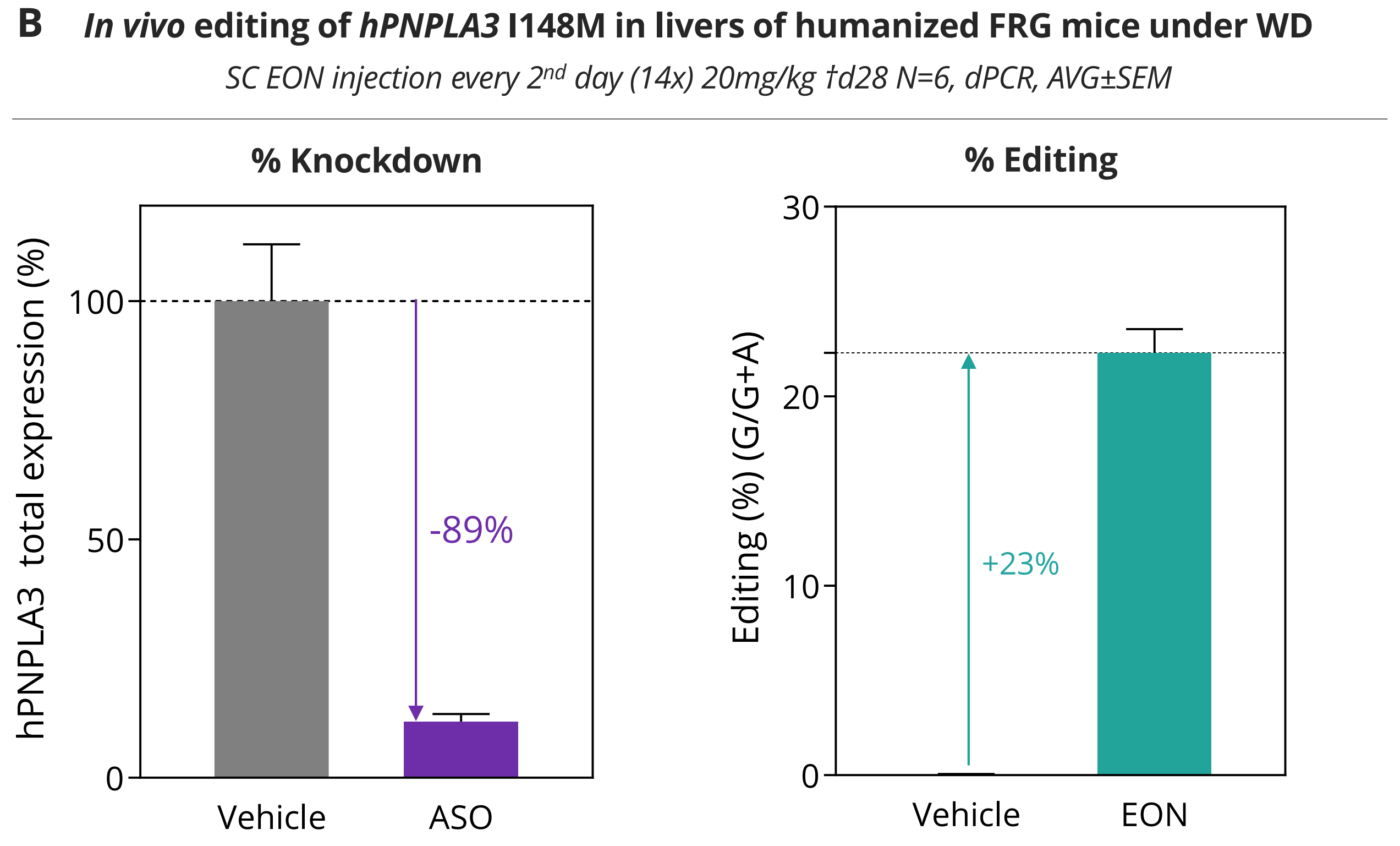
Figure 4. Editing efficiency of PNPLA3 in I148M expressing PHH. Treatment conditions: PNPLA3 EON 5 μM, gymnosis, 72h treatment, n=4, dPCR, mean, SD.

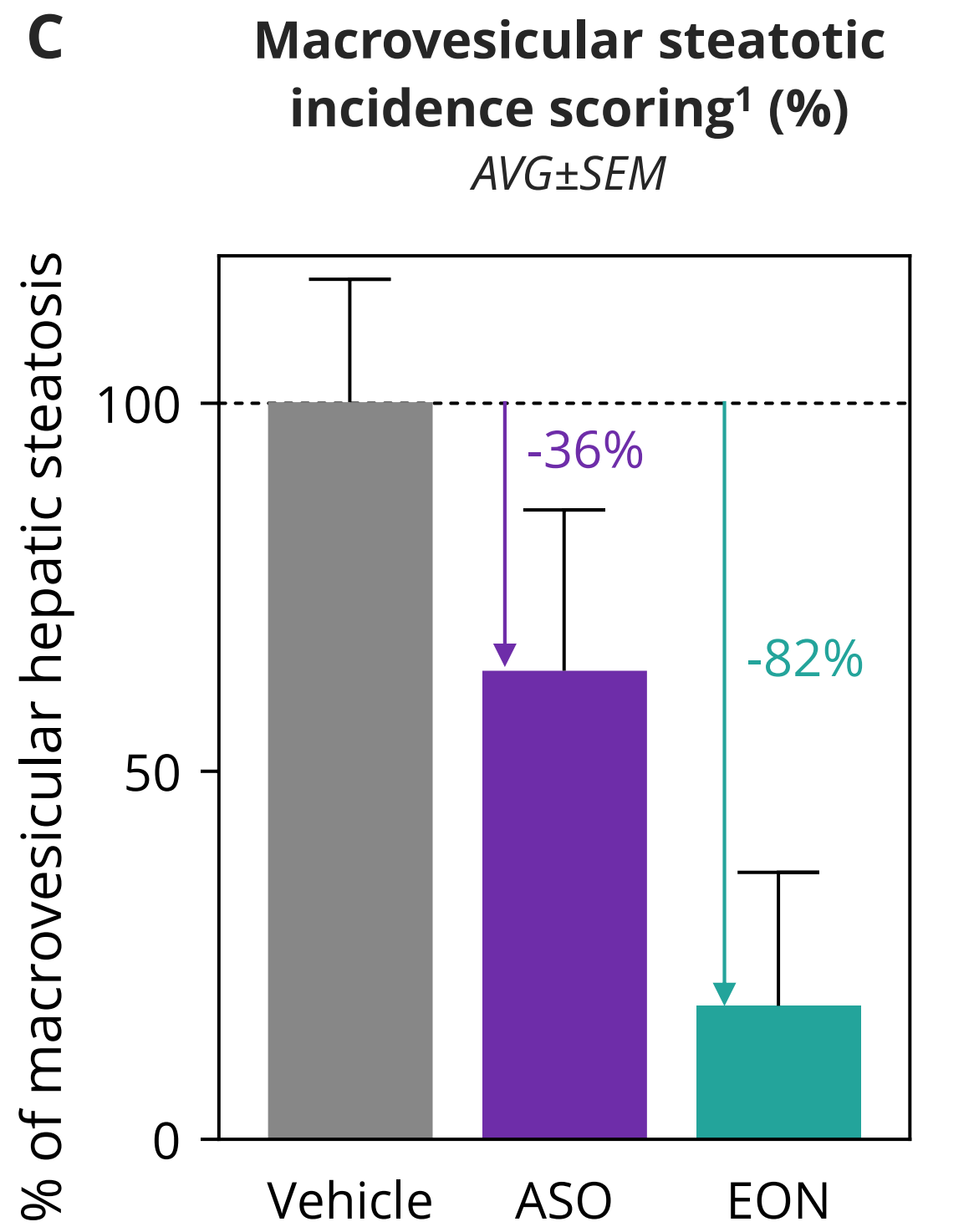
PNPLA3 I148M editing reduces steatotic burden *in vivo*

To assess whether RNA editing–mediated conversion of *PNPLA3* 148M to 148V can reverse liver steatosis, FRG mice engrafted with human PNPLA3 I148M PHHs were fed a western diet (WD) for 4 weeks to induce early-stage MASLD. Mice were subsequently treated with EON 5, via subcutaneous dosing (20 mg/kg, every 2 days) for 28 days while maintaining WD exposure (**Fig. 5A**). A clinical-stage gapmer ASO was included as a comparator to evaluate RNA editing versus transcript knockdown. Following treatment, liver RNA was analyzed to quantify *PNPLA3* editing efficiency, and histological sections were blindly scored for macrovascular lipid droplets (MVLD). No changes in body weight or liver histopathology were observed. Mean editing efficiency reached ~23%, whereas the ASO achieved ~90% knockdown (**Fig. 5B**).

Notably, EON 5 treatment reduced MVLD by 82%, compared to 36% in the ASO group (**Fig. 5C**). These results demonstrate that RNA editing of *PNPLA3* I148M can reverse hepatic steatosis and suggest that preserving PNPLA3 function is more effective than complete transcript knockdown.







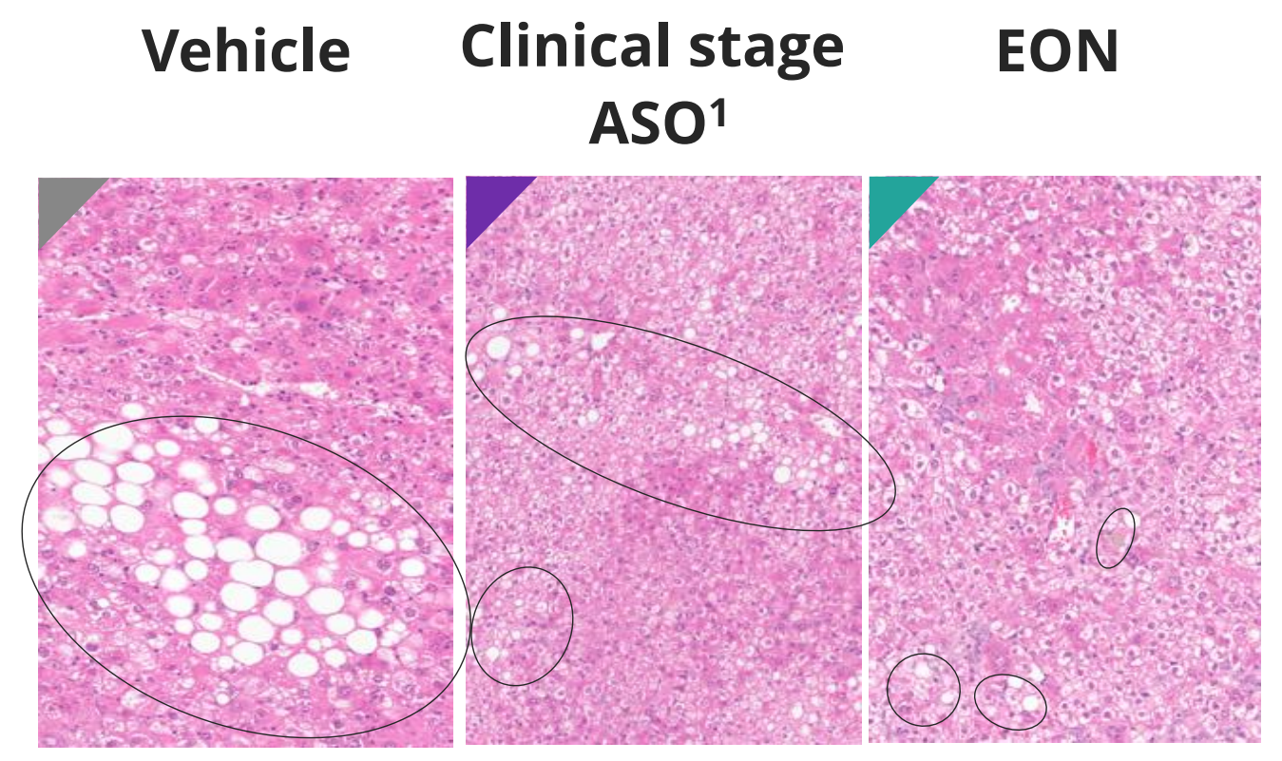


Figure 5. (**A**) PoM dosing regime. (**B**) hPNPLA3 I148M Editing and Knockdown in liver of FRG mice engrafted with PNPLA3 I148M expressing donor PHHs under WD. (**C**) Macrovescular stetaotic incidence scoring (%) in liver section.

Conclusions

- **Axiomer-mediated RNA editing efficiently converts PNPLA3 I148M in human hepatocytes in vitro and in vivo**
- **Editing translates into significant reduction of hepatic steatosis in a humanized MASLD model**
- **Despite lower molecular correction, RNA editing outperforms ASO-mediated knockdown in reducing lipid accumulation**
- **These data support restoration of PNPLA3 function as a superior therapeutic strategy over gene silencing**
- **Axiomer RNA editing represents a promising therapeutic approach for MASLD/MASH patients carrying PNPLA3 I148M**

Literature

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