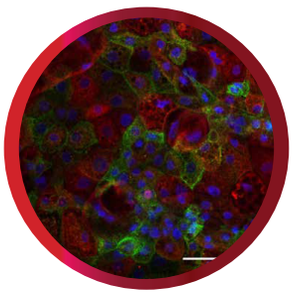
DefiniGEN

DefiniGEN human iPS-derived NAFLD hepatocytes



Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of fat within the liver that can lead to inflammation, fibrosis, and hepatocellular carcinoma. The most common disease implicated genetic variant is I148M in the gene coding for Patatin-like phospholipase domain-containing protein 3 (PNPLA3). DefiniGEN disease modelled NAFLD hepatocytes represent an optimized model for drug discovery applications and a principal tool for elucidating the underlying mechanisms of the disease.

Disease circuit verification

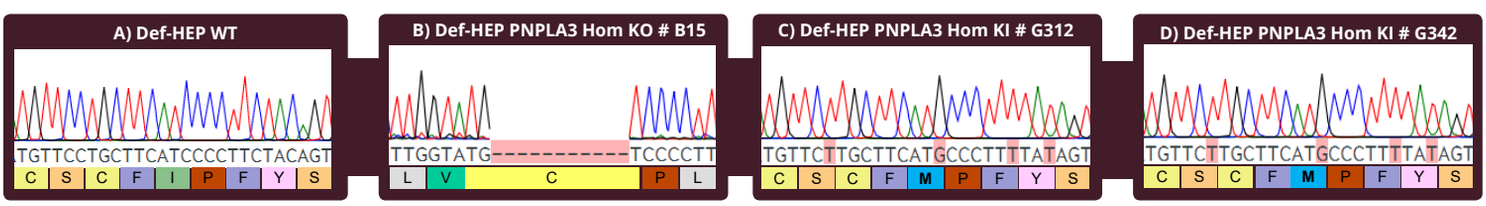


Figure 1. Sanger sequencing of the edited PNPLA3 gene using CRISPR/Cas9 in the reference hiPSC line. (A) PNPLA3 gene in wild-type line. **(B)** PNPLA3 homozygous knock-out clone with 11bp deletion causing a frameshift mutation. **(C,D)** PNPLA3 homozygous knock-in clones with I148M mutation (Isoleucine to methionine at position 148, exon 3 (I148M, rs738409).

Immunofluorescence analysis

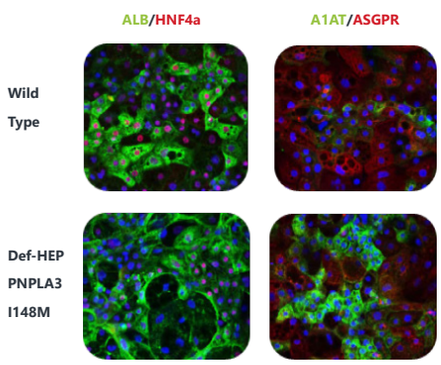


Figure 2. Immunofluorescence staining for hepatocyte-specific markers in Def-HEP PNPLA3 cells. Immunofluorescence analyses for expression of general hepatocyte maturation markers including ALB, HNF4a, A1AT and ASGPR.

Fatty acid accumulation

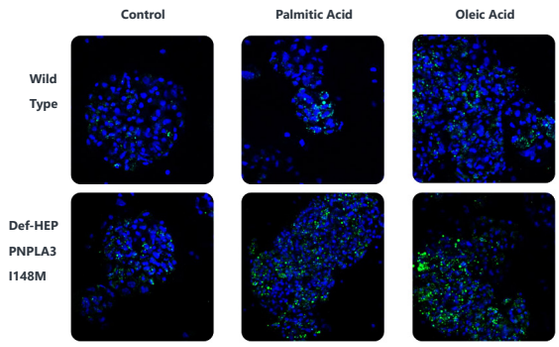


Figure 3. Fatty acid accumulation in Def-HEP PNPLA3 cells. When cells are treated with 0.25 mM of either oleic acid or palmitic acid, the fatty acids are absorbed by the hepatocyte-like cells and accumulated into lipid droplets within the cells. After treatment, the lipid droplets were stained with BODIPY. The I148M variant cells demonstrated increased fatty acid accumulation upon treatment with these fatty acids in comparison with the isogenic wild-type control.

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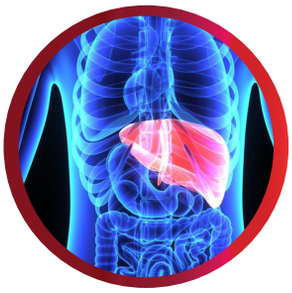

DefiniGEN custom NAFLD disease model development

Custom NAFLD model development

Patient derived model	CRISPR gene-edited model
Recapitulating patient genetics and phenotype in the cell model, complete with patient clinical data	Systematically introducing mutations into specified NAFLD associated-loci in Def-HEP cells and providing the isogenic control for screening programmes (manipulation at multiple loci can also be achieved)

Subset of potential causal gene variants have been determined which are currently a significant focus on NAFLD studies

NAFLD gene variants

PNPLA3 rs738409 impaired hepatocellular triglycerides hydrolysis and increased lipogenesis associated to the 148 M allele

GCKR rs1260326 increased glycolysis favours an increase in triglyceride levels

TM6SF2 rs58542926 impaired mobilization of neutral lipids for very low-density lipoprotein (VLDL) assembly and secretion by the liver in E167K carriers

MBOAT7 rs641738 variant causing decreased MBOAT7 expression, predisposes to NAFLD/NASH by affecting the acyl remodelling of phosphatidylinositol in the liver

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