DefiniGEN
DISEASE MODEL INNOVATION

Disease Modeling

DefiniGEN human iPSC-derived
Wilson's disease modelled hepatocytes

Description

Wilson's disease (WD) is an autosomal recessive disorder of copper metabolism with a worldwide prevalence of 1/30,000 to 1/100,000. It is caused by loss-of-function mutations in the ATP7B gene, which encodes copper-transporting P-type ATPase, expressed primarily in the liver. ATP7B mutations result in defective copper homeostasis and excessive copper accumulation predominantly in the liver and brain, leading to a series of metabolic disorders in the liver and nervous system.

Characterization

DefiniGEN have created Wilson's disease model hepatocyte for the evaluation of therapeutic candidates.

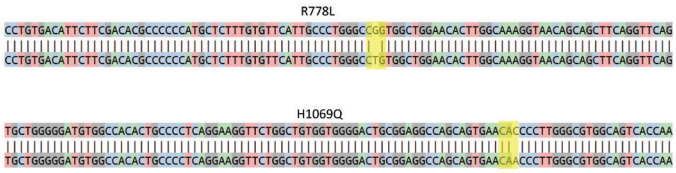

Figure 1. Sanger sequencing analysis showing the presence of a homozygous c.2333G>T (CGG>CTG; R778L) mutation in exon 8 of ATP7B gene in WD R778L iPSC model (top) and a homozygous c.3207C>A (CAC>CAA; H1069Q) mutation in exon 14 of ATP7B gene in the WD H1069Q iPSC model (bottom). The rectangles indicate the missense pathogenic mutations.

Advantages

Disease circuit verified Wilson's disease is caused by mutations in the ATP7B gene

Display multiple key hepatocyte markers A1AT, Albumin, Glucose

Optimized bioassay measuring oxidative stress as an end-point in Hepatocyte-like cells (HLCs)

Application for the preclinical screening of small molecules, siRNA and oligonucleotide therapeutic candidates and base editing approaches

Standardized cell product containing iPSC-derived human hepatocytes producing reproducible and biologically relevant data

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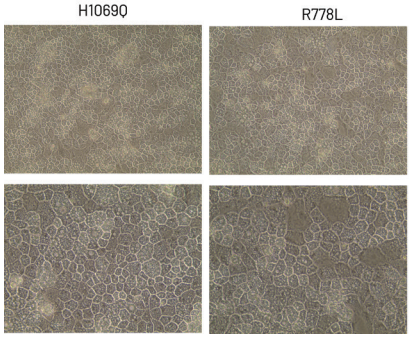

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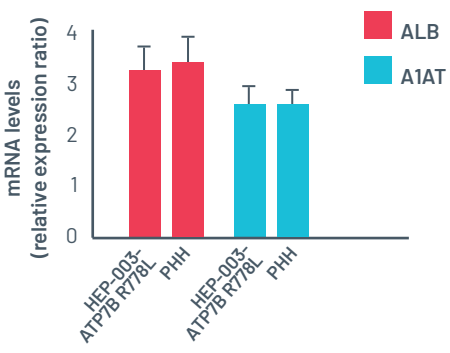

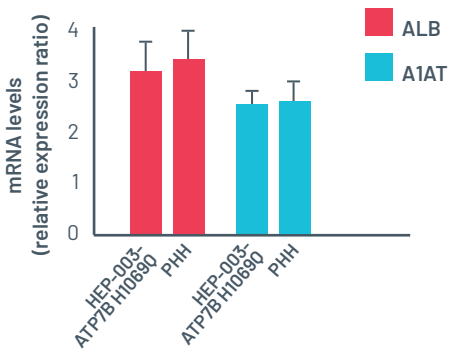

Figure 2 A). Representative images of ATP7B R778L and ATP7B H1069Q HEPs showing a typical cobblestone morphology. B) Gene expression analysis of key hepatic markers: ALB and A1AT, showing efficient hepatic differentiation ability of WD-introduced iPSCs. PHH cDNA used as the positive control of full hepatic maturation.

Phenotypic validation

Wilson's Disease (WD) can be modelled measuring oxidative stress as an endpoint in HLCs

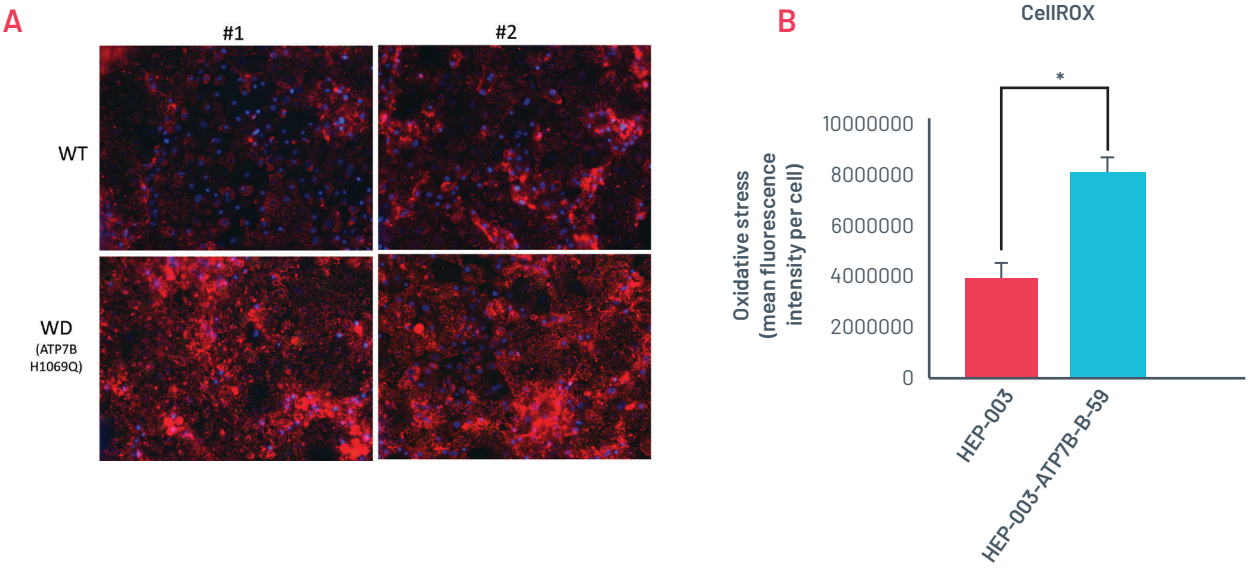

Figure 3. A) Staining with CellROX Orange in wild-type (HEP-003) and H1069Q Wilson's Disease (WD) HLCs (HEP-003-ATP7B-H1069Q) when challenged with 250µM $CuCl_2$ for 24h. Representative images of n=3 independent experiments. B) Quantification of CellROX Orange staining in wild-type and H1069Q WD HLCs (n=3 independent experiments). * $p<0.05$

WD HLCs show oxidative stress consistently upon being challenged with $CuCl_2$

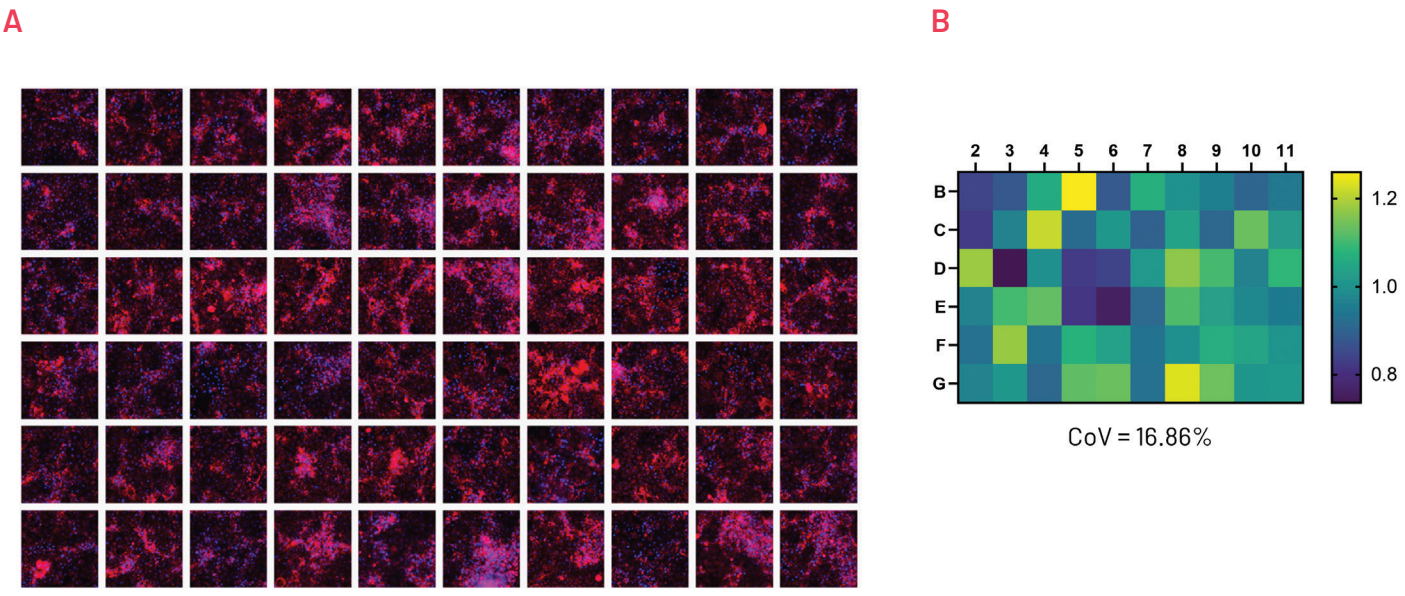

Figure 4. A) Staining with CellROX Orange shows the levels of oxidative stress in WD H1069Q HLCs HLCs across the central wells of a 96 well plate when treated with $CuCl_2$ for 24h. Representative images of 3 independent experiments. B) Quantification of the CellROX Orange staining across the central wells of a 96 well plate. Values normalized to average of the plate set as 1 (n=3).

WD HLCs respond to reference chelator Trientine Hydrochloride

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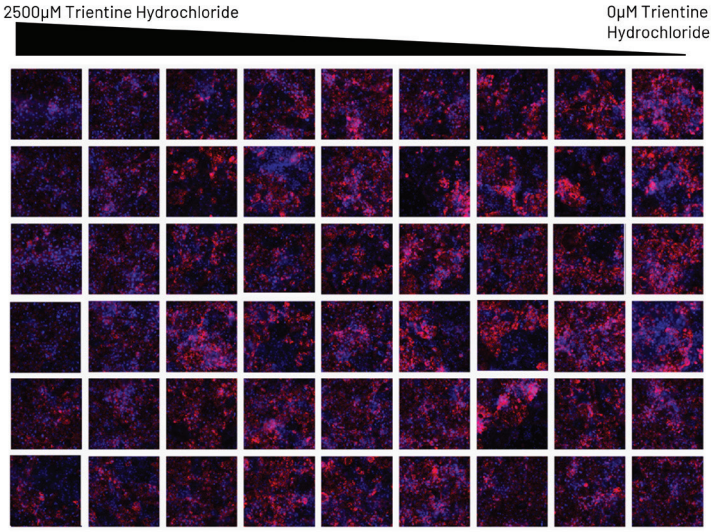

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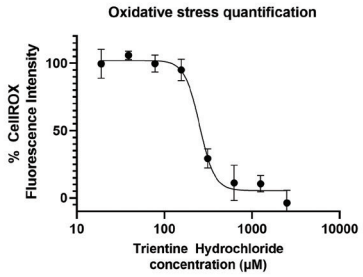

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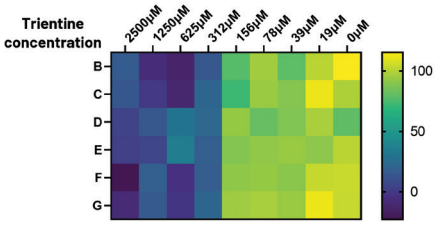

Figure 5. A) CellROX Orange staining showing levels of oxidative stress in H10690 WD HLCs treated with Trientine Hydrochloride in a dose-dependent manner. B) Quantification of the oxidative stress showing normalised MFI per cell. Results are normalized to vehicle-treated cells set as 100% presented as mean±SEM of n=3 biological replicates C) Heatmap showing quantification of oxidative stress upon treatment with decreasing concentrations of Trientine Hydrochloride.

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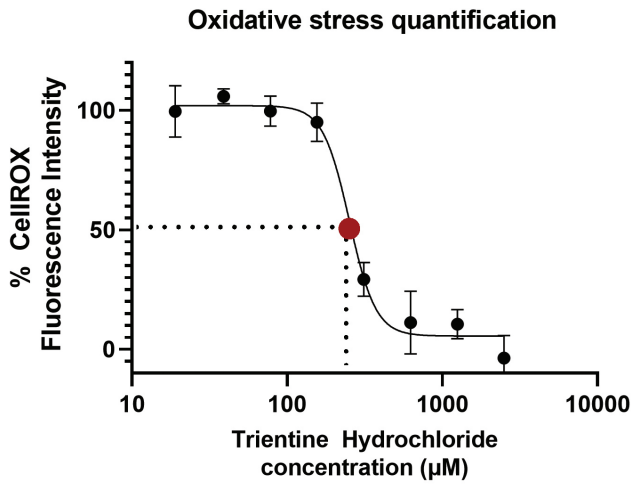

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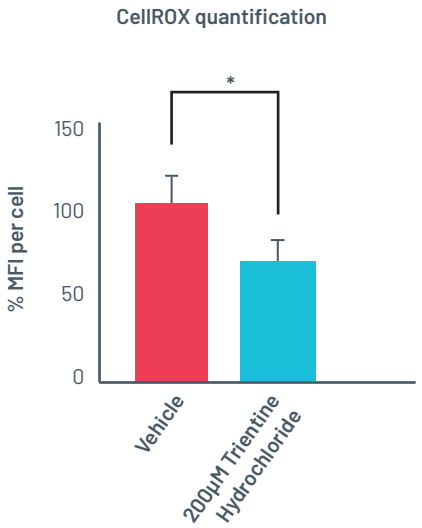

Figure 6. A) Quantification of oxidative stress levels in H10690 WD HLCs upon treatment with increasing concentrations of Trientine Hydrochloride show an EC50 close to 200µM (n=3). B) H10690 WD HLCs oxidative stress response to treatment with 200µM of Trientine Hydrochloride. Results expressed as mean±SEM of n=3 biological replicates. *p<0.05

Phenotype of H1069Q WD HLCs can be recovered with WT ATP7B mRNA

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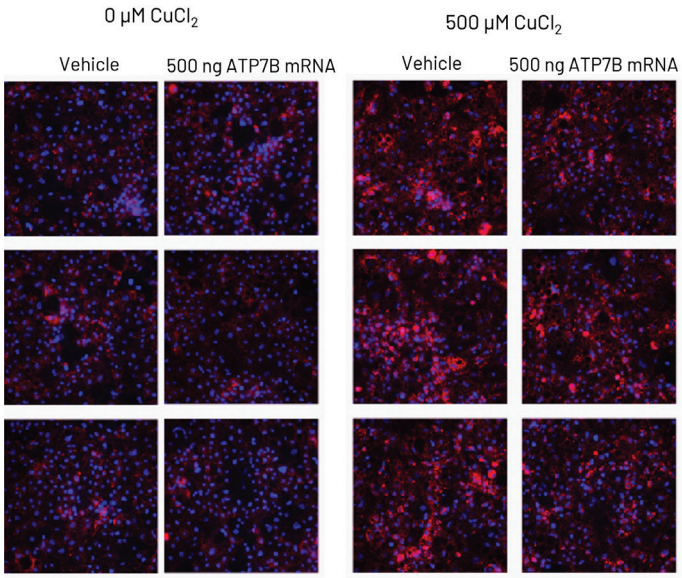

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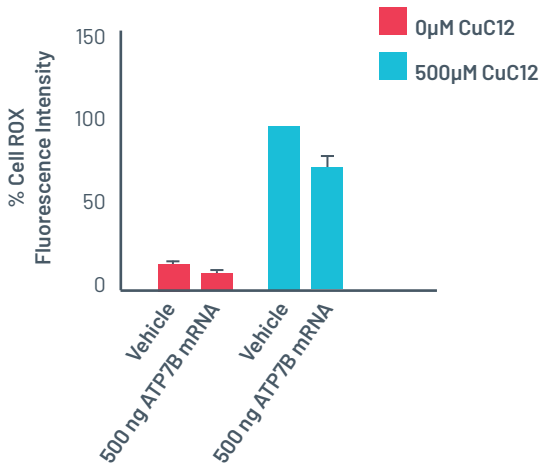

Figure 7. A) CellROX Orange staining showing levels of oxidative stress in in H1069Q WD HLCs upon transfection with WT ATP7B mRNA for 96h. B) Quantification of the oxidative stress showing normalised MFI per cell. Results normalised to 500µM CuCl2 + vehicle set as 100% and expressed as mean±SEM of n=3 biological replicates

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