DefiniGEN
DISEASE MODEL INNOVATION

Disease Modeling

DefiniGEN human iPSC-derived Alpha-1 Antitrypsin Deficiency disease modelled hepatocytes

Description

Alpha-1-antitrypsin deficiency (A1ATD) is a disease which manifests in the liver as a result of an inherited mutation in the SERPINA1 gene (E342K ZZ being the most prevalent) and results in the misfolding of the A1AT protein, forming aggregates in the endoplasmic reticulum of the hepatocytes thereby causing liver damage. In addition, the lack of active A1AT protein in the bloodstream causes a failure to inhibit neutrophil elastase in the lungs, leading to chronic and life-threatening lung damage.

Characterization

DefiniGEN have an iPSC line which has been selected out of many hundreds of lines for its robust differentiation characteristics, and, by using CRISPR gene editing, have generated a ZZ mutant with an otherwise healthy genetic background for primary screening activities.

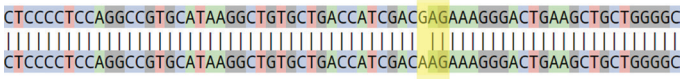

Figure 1. Sanger sequencing showing homozygous E342K mutation (GAG > AAG) in the SERPINA1 gene. The codon change is highlighted in yellow. The wild type sequence (Ref Seq) is shown at the top while the mutant line at the bottom.

Advantages

Disease circuit verified Alpha-1 antitrypsin deficiency is the result of an inherited mutation in the SERPINA1 gene.

Display multiple key hepatocyte markers A1AT, Albumin, Glucose

Optimized bioassay directly measures hepatic polymer accumulation which is also used as a clinical endpoint for the *in-vivo* testing of candidate therapeutics.

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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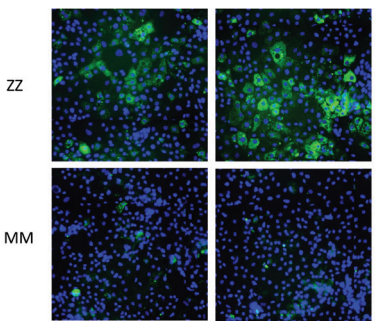

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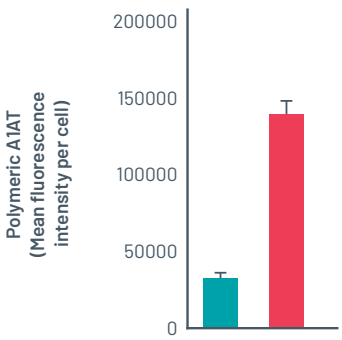

Figure 2. A) Immunostaining with polymer-specific A1AT antibody shows intracellular accumulation of polymeric A1AT in ZZ hepatocyte-like cells (HLCs) vs. isogenic MM HLCs. B) Quantification of the intracellular levels of polymeric A1AT in ZZ hepatocyte-like cells (HLCs) vs. isogenic MM HLCs. Mean fluorescence intensity (MFI) was normalised to number of nuclei. Results presented as mean ± SEM of n=3 independent experiments.

Phenotypic validation

Polymer build up in the livers of A1ATD patients, and DefiniGEN's model directly measures hepatic polymer accumulation which is also used as a clinical endpoint for the *in-vivo* testing of candidate therapeutics.

An optimized immunofluorescent bioassay for polymeric A1AT gives a reliable endpoint, and treatment windows for different molecular entities have been established.

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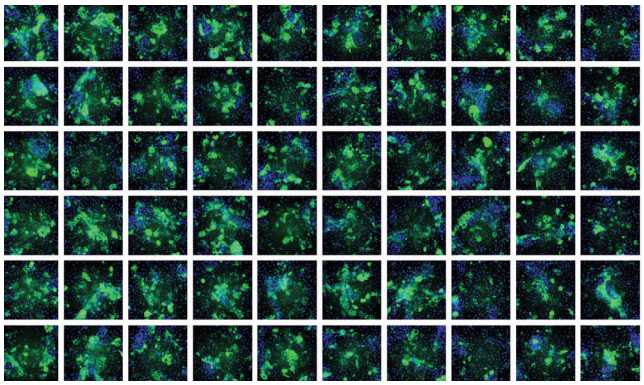

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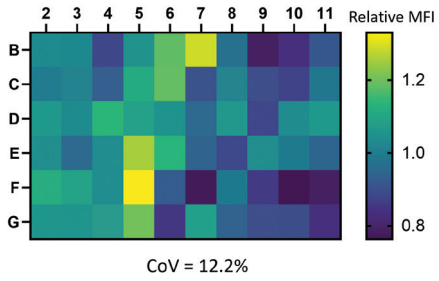

Figure 3. Immunostaining with polymer-specific A1AT antibody shows levels of polymeric A1AT in ZZ-HLCs across the central wells of a 96 well plate. Representative images of 3 independent experiments. B) Quantification of the intracellular levels of polymeric A1AT in in ZZ-HLCs across the central wells of a 96 well plate relative to the average of the plate set as 1. Results expressed as average from n=3 independent experiments.

Phenotypic recovery

Autophagy-inducing compounds including carbamazepine have been shown to accelerate the clearance of polymeric A1AT in hepatocytes, and DefiniGEN have used this compound as our standard positive control in every plate run for small molecule screens.

Recent advances in gene therapies and innovative small molecule approaches offer new hope for curative treatments, however there is a paucity of pre-clinical human models available to assess efficacy. DefiniGEN offer screening services on metabolically competent hepatocytes with a mature phenotype, to help support drug development for this indication and generate valuable human data for your candidate therapeutics. Our cells give robust responses to carbamazepine and other autophagy inducing small molecules.

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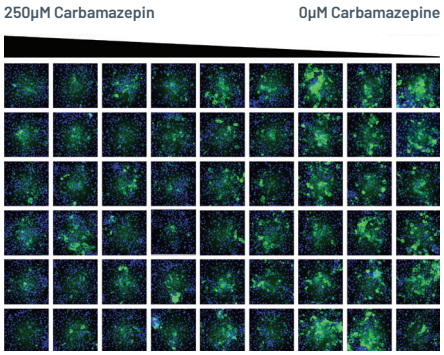

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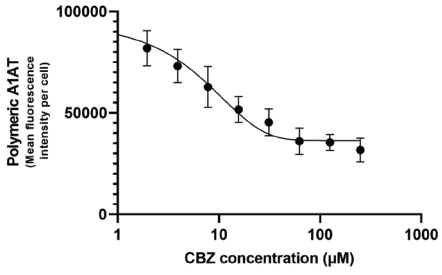

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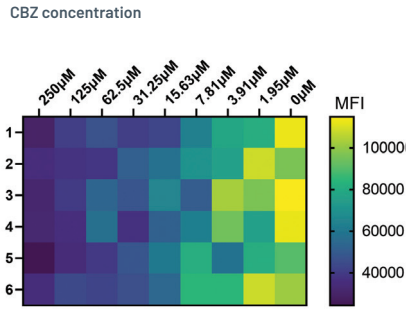


Figure 4. A) Intracellular accumulation of polymeric A1AT in ZZ-HLCs in response to treatment with different concentrations of Carbamazepine following immunostaining with polymer-specific A1AT antibody. B) Quantification of the intracellular levels of polymeric A1AT showing MFI per cell upon treatment with CBZ. Results expressed as mean±SEM of n=4 independent experiments. C) Heatmap showing quantification of polymeric A1AT across a 96 well plate upon treatment with decreasing concentrations of CBZ, representative of 4 independent experiments.

4.1 Phenotype Recovery using autophagy compounds

A1ATD HLCs respond to novel autophagy-inducing compounds

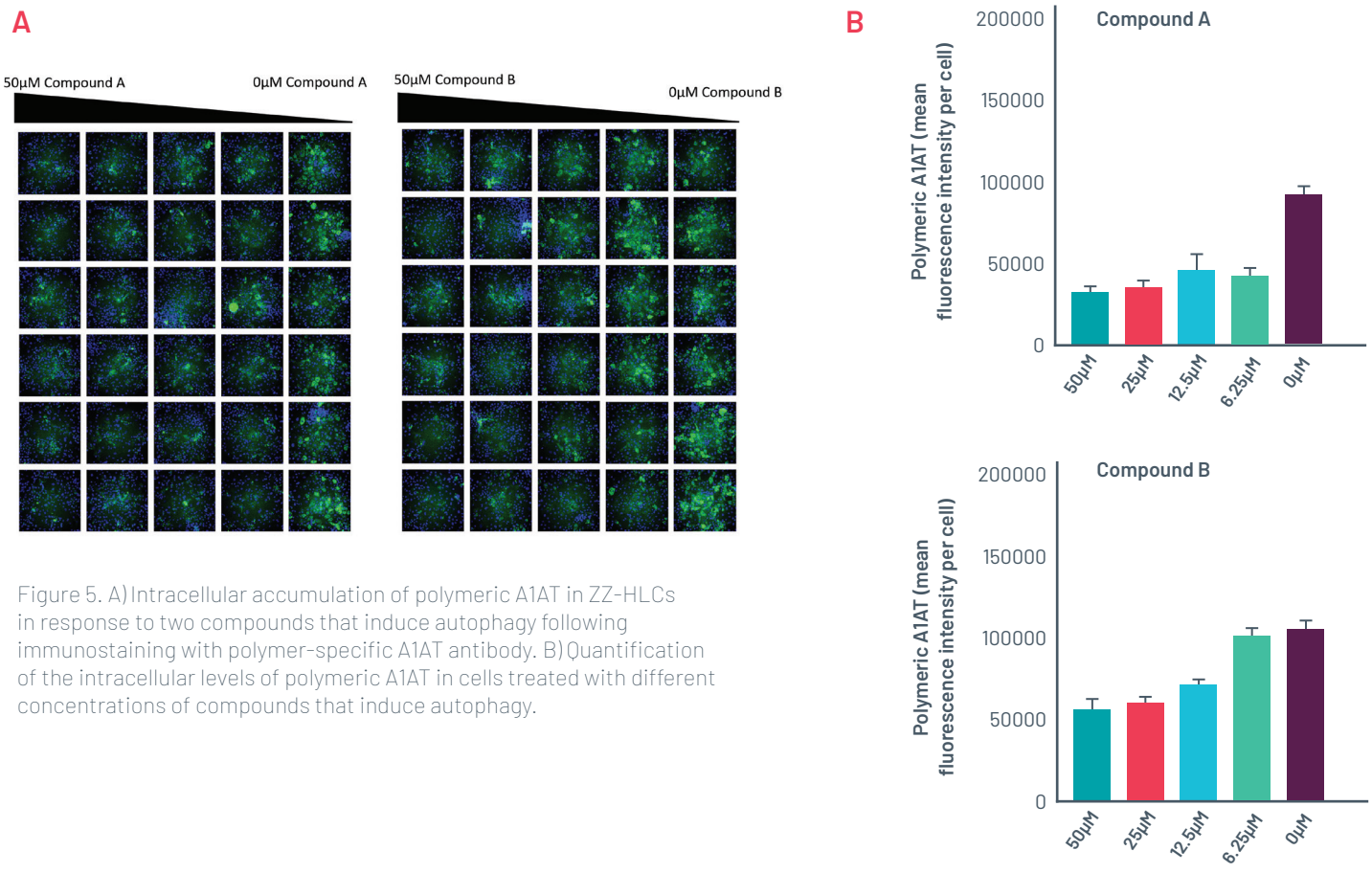


Figure 5. A) Intracellular accumulation of polymeric A1AT in ZZ-HLCs in response to two compounds that induce autophagy following immunostaining with polymer-specific A1AT antibody. B) Quantification of the intracellular levels of polymeric A1AT in cells treated with different concentrations of compounds that induce autophagy.

4.2 Phenotypic recovery using Nucleotide based therapeutics

Def-HEP A1ATD cells are readily transducible or transfectable, and lend themselves to the evaluation of gene therapy approaches, from siRNA to viral vectors or base-editing approaches. Our clients can send their compounds or reagents directly to our facility in Cambridge UK and data can typically be generated in 6-8 weeks.

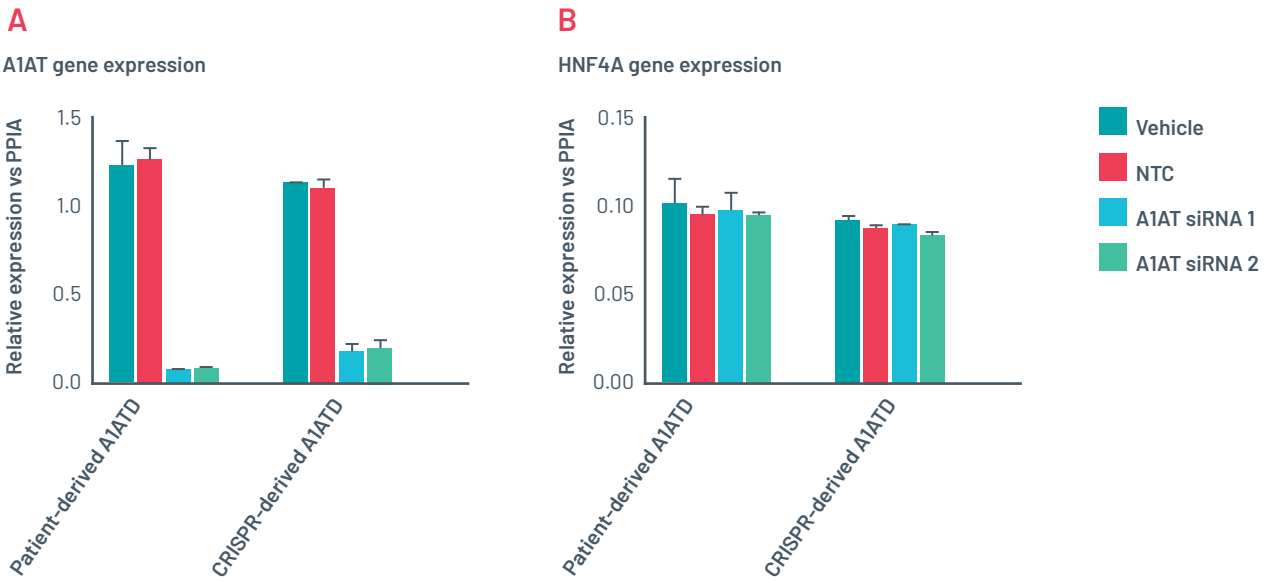
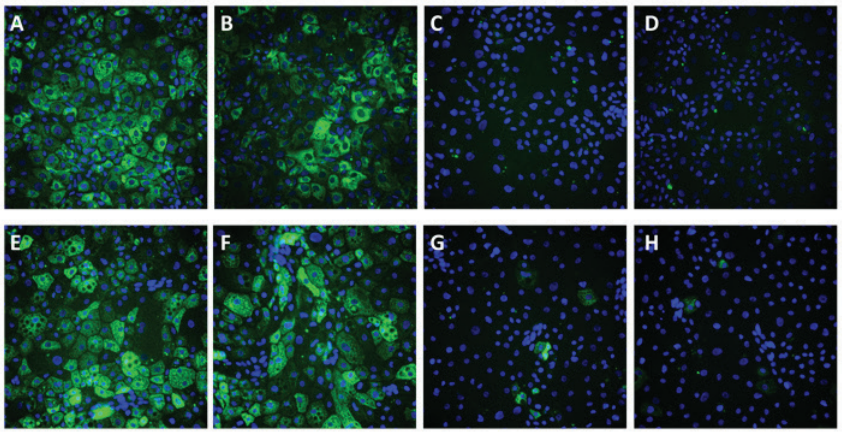


Figure 6. Gene expression of Def-HEP A1ATD 48h after transfection with scramble siRNA (NTC) and two different combinations of siRNA targeting SERPINA1 (A1AT). Gene expression of A1AT dramatically decreases with both sets of siRNAs (A) but other genes such as HNF4A (B) remain consistently expressed.



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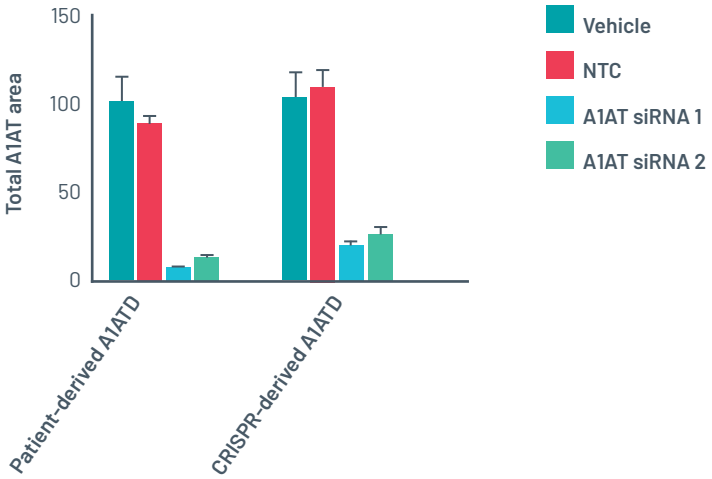

Figure 7. Immunofluorescence for polymeric A1AT. Representative pictures of polymeric A1AT staining for patient derived A1ATD hepatocyte-like cells transfected for 72h with Vehicle (A), Scrambled siRNA (B), and two different combinations of siRNA targeting SERPINA1 (C, D). Representative pictures of A1AT staining for CRISPR-derived A1ATD hepatocytes transfected for 72h with Vehicle (E), Scrambled siRNA (F), and two different combinations of siRNA targeting SERPINA1 (G, H). (I) Quantification of polymeric inclusions of A1AT per cell in different conditions.

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