DefiniGEN
DISEASE MODEL INNOVATION

Hepatocyte-like cell functionality

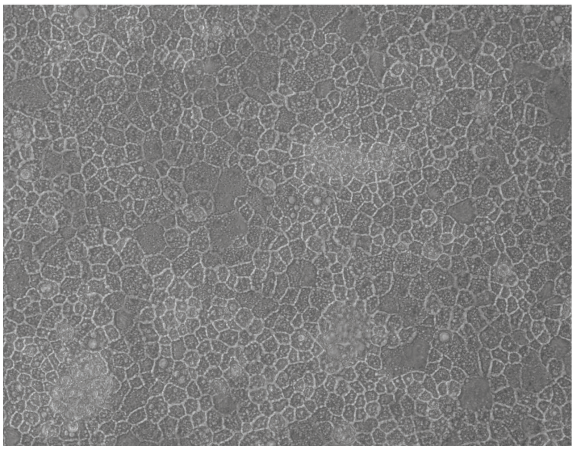
Description

DefiniGEN's proprietary differentiation protocol permits large-scale generation of HLCs with field leading purity and functionality. Importantly, the HLCs successfully recapitulate key aspects of disease pathophysiology across a wide- range of conditions that affect different aspects of liver function.

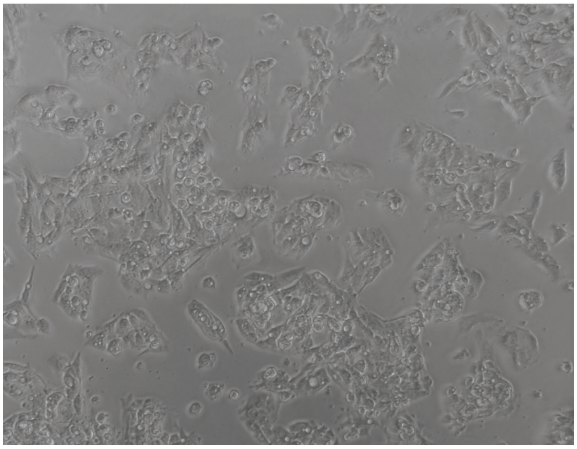
Characterization

DefiniGEN hepatocyte-like cells (HLCs) demonstrate characteristic hepatocyte cobblestone morphology.

HLCs



HepG2



Advantages

Demonstrate characteristic hepatocyte cobblestone morphology

Express higher levels of liver maturity markers compared to liver carcinoma cell lines and comparable to primary human hepatocytes (PHH)

Express higher levels of urea cycle markers and secrete higher levels of urea compared to liver carcinoma cell lines

Demonstrate higher levels of P450 markers compared to liver carcinoma cells and functional CYP3A4 activity and induction

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

Figure 1. DefiniGEN hepatocyte-like cells (HLCs) demonstrate the characteristic cobblestone morphology and the presence of a uniformed monolayer following >3 weeks of iPSC differentiation, compared to liver carcinoma HepG2 cells. Objective: 10x

Maturity marker analysis

DefiniGEN HLCs express higher levels of liver maturity markers compared to liver carcinoma cell lines and comparable to primary human hepatocytes (PHH).

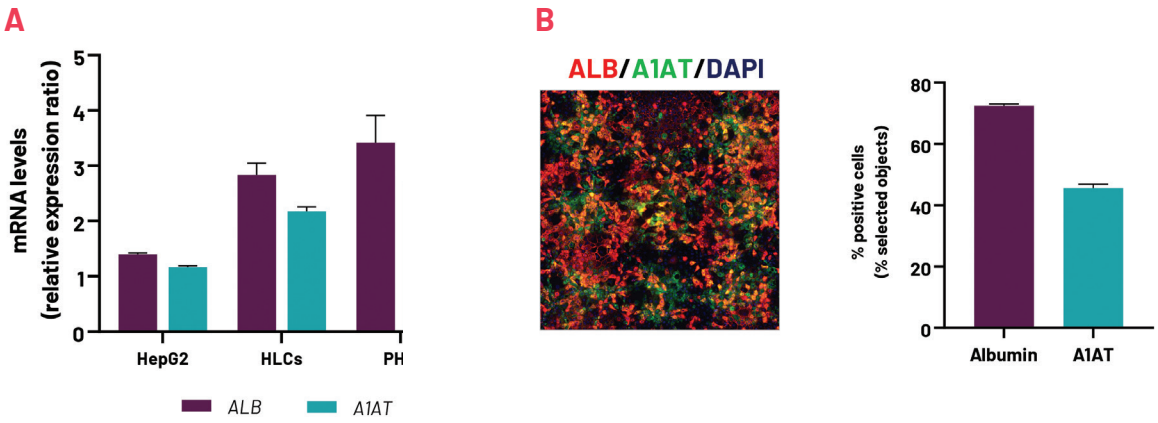


Figure 2. A) mRNA expression levels of albumin (ALB) and alpha-1-antitrypsin (A1AT) in liver carcinoma HepG2 cells, DefiniGEN HLCs, and primary human hepatocytes (PHH). B) Representative picture of DefiniGEN HLCs revealing protein expression of the hepatocyte maturity markers albumin (red) and alpha-1-antitrypsin (green) by immunocytochemistry (ICC). Data are presented as mean±SD of n=3-4 independent experiments. mRNA expression data were normalised to PPIA and ICC to total nuclei number. Objective: 10x.

Urea cycle marker analysis

DefiniGEN HLCs express higher levels of urea cycle markers and secrete higher levels of urea compared to liver carcinoma cell lines.

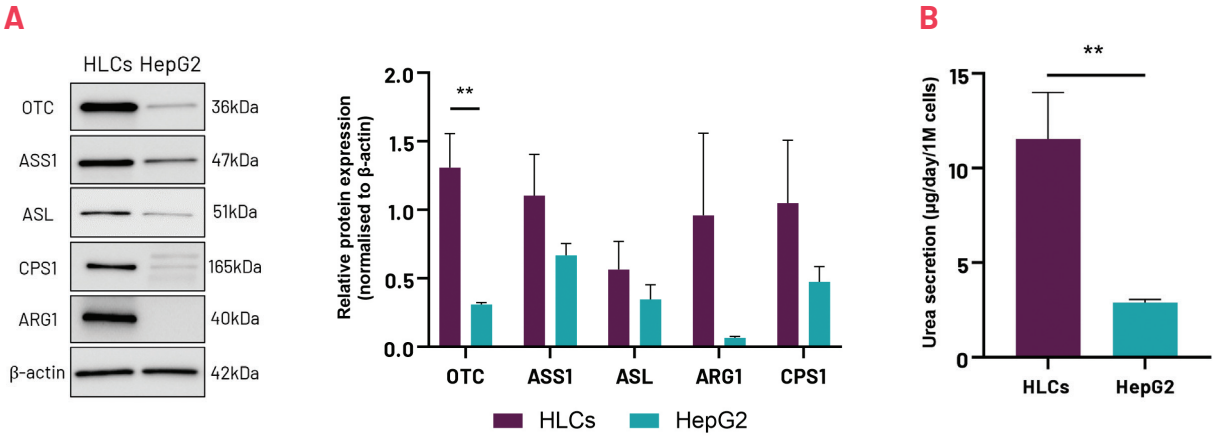


Figure 3.A) Protein expression levels of the urea cycle enzymes OTC, ASS1, ASL, CPS1, and ARG1 in liver carcinoma HepG2 cells and DefiniGEN HLCs. B) Urea secretion in DefiniGEN HLCs and liver carcinoma HepG2 cells. Data are presented as mean±SD of n=3-4 independent experiments. Protein expression data were normalised to beta actin. Urea secretion data were normalised to total cell number.

Functional gluconeogenesis pathway

DefiniGEN HLCs demonstrate functional gluconeogenesis pathway and respond to hormonal stimulation.

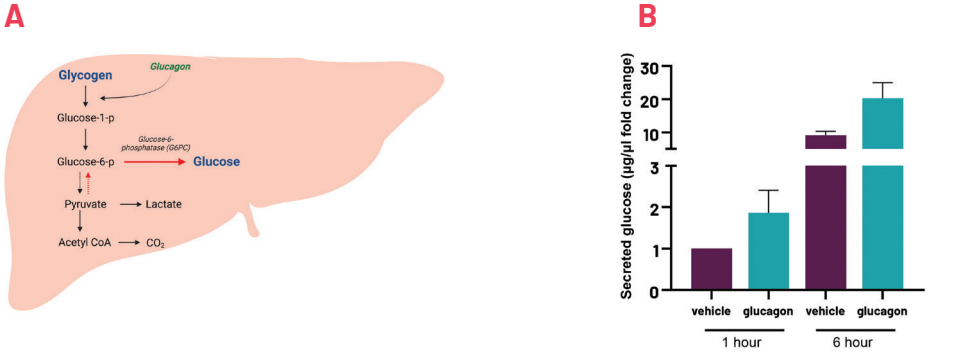


Figure 4. A) Simplified schematic on the gluconeogenesis/glycogenolysis pathways within human liver. B) Media glucose levels from DefiniGEN HLCs treated with glucose-free media for 1h and 6h with or without glucagon, suggesting the de novo synthesis of glucose from non-lipid precursors, accompanied by functional glycogenolysis pathway additionally contributing to total glucose levels. Data are presented as mean±SD of n=2 technical replicates.

P450 marker analysis

DefiniGEN HLCs demonstrate higher levels of P450 markers compared to liver carcinoma cells and functional CYP3A4 activity and induction.

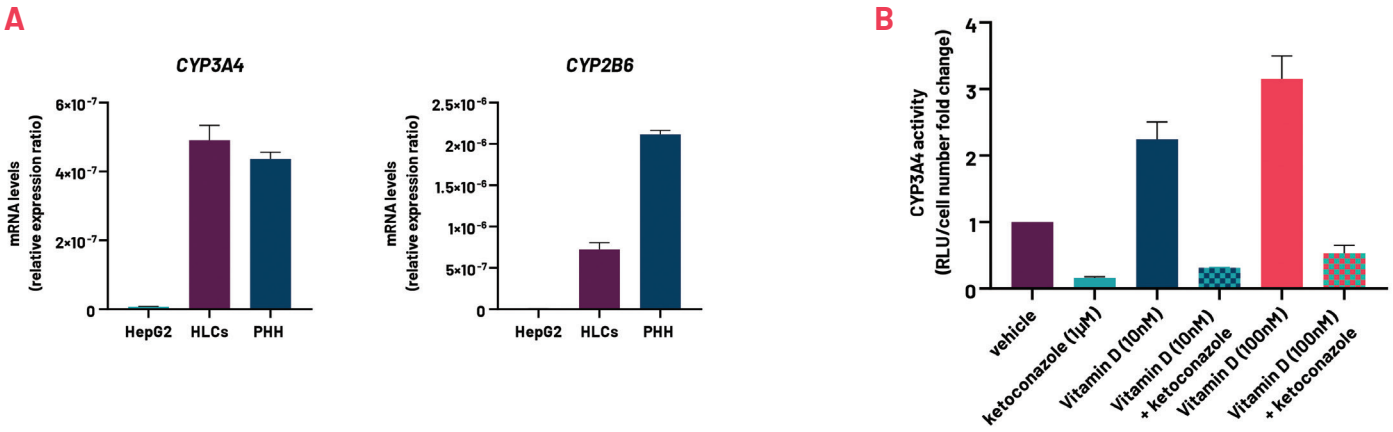


Figure 5. A) mRNA expression levels of CYP3A4 and CYP2B6 in liver carcinoma HepG2 cells, DefiniGEN HLCs, and primary human hepatocytes (PHH). B) CYP3A4 induction in DefiniGEN HLCs following 72h treatment with vehicle, 1μM ketoconazole (CYP3A4 inhibitor), 10-100nM vitamin D (CYP3A4 inducer), or a combination of vitamin D and ketoconazole. mRNA data are presented as mean±SEM of n=2-3 independent experiments and were normalised to 18SrRNA. CYP3A4 activity data are presented as mean±SD of n=2 independent experiments and were normalised to total cell number.

Modalities

DefiniGEN HLCs can be a suitable cell platform for a variety of non-viral nucleic-acid based modalities.

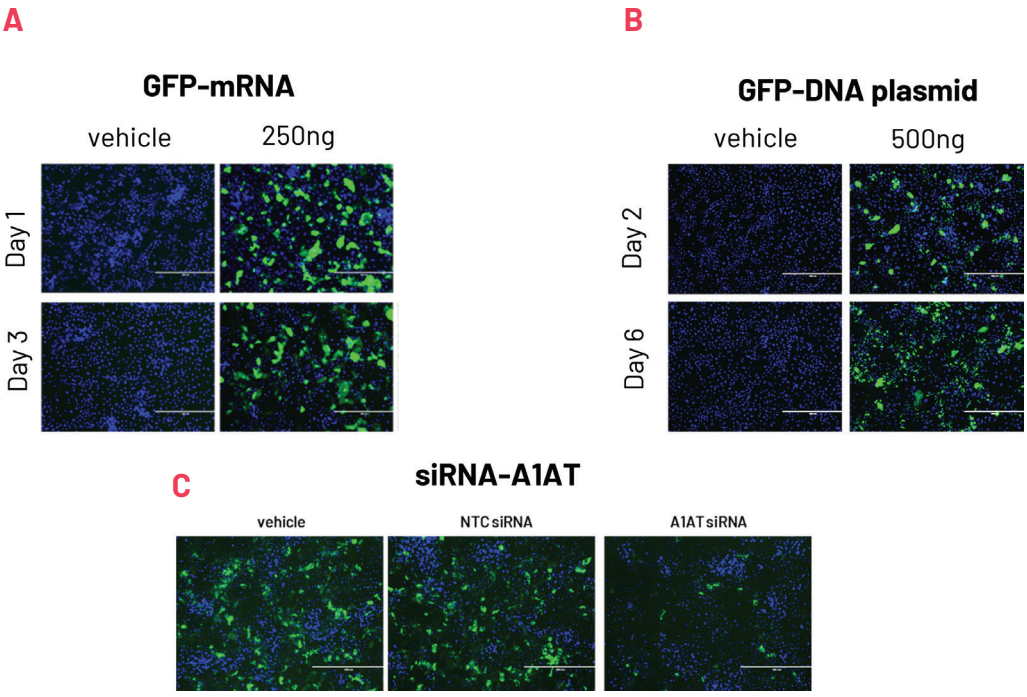


Figure 6. GFP or A1AT expression in DefiniGEN HLCs following transfection with: A) 250ng of GFP-mRNA for 1 and 3 days, B) 500ng of GFP-DNA for 2 and 6 days, C) 50nM of either anti-SERPINA1 siRNA or non-template control (NTC) siRNA for a period of 6 days. Scale bar: 400μm

Modalities

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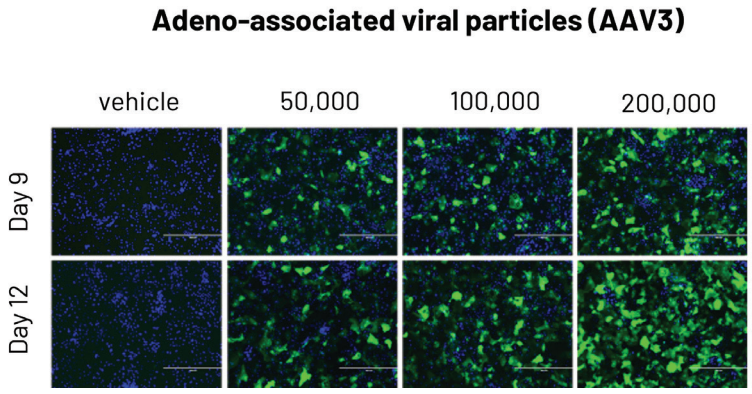
A



B

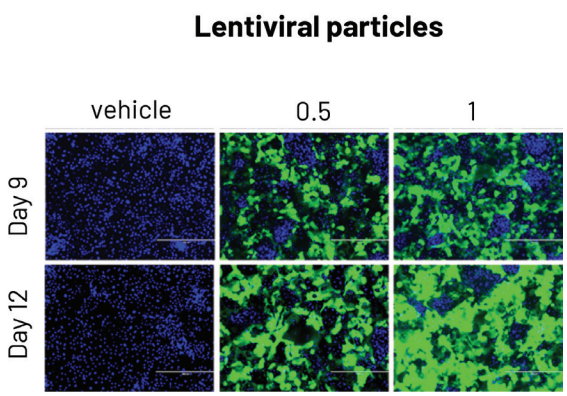


Figure 7. GFP expression in DefiniGEN HLCs following transduction with: A) increasing MOI (0-200,000) of GFP-AAV (Serotype 3), 9-, and 12-days post-transduction, B) increasing MOI (0-1) of GFP-lentivirus (LV) 3-, 6-, 9-, and 12-days post-transduction. Scale bar: 400µm

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