DefiniGEN
DISEASE MODEL INNOVATION

Opti-HEP Functionality

DefiniGEN's proprietary differentiation protocols permit the large-scale generation of iPSC-derived hepatocytes (Opti-HEP) with field-leading purity and functionality. Importantly, Opti-HEP successfully recapitulate key aspects of disease pathophysiology across a wide range of conditions that affect different aspects of liver function.

Advantages

» **Demonstrate characteristic** hepatocyte cobblestone morphology

» **Express comparable levels of liver maturity markers** to primary human hepatocytes

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

» **Demonstrate comparable levels of CYP450 markers and CYP3A4 activity** to primary human hepatocytes

» **Demonstrate functional localization and function of ASGR1** for GalNAc-dependent drug deliveries

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

Morphology

DefiniGEN Opti-HEP demonstrate the characteristic hepatocyte cobblestone morphology.

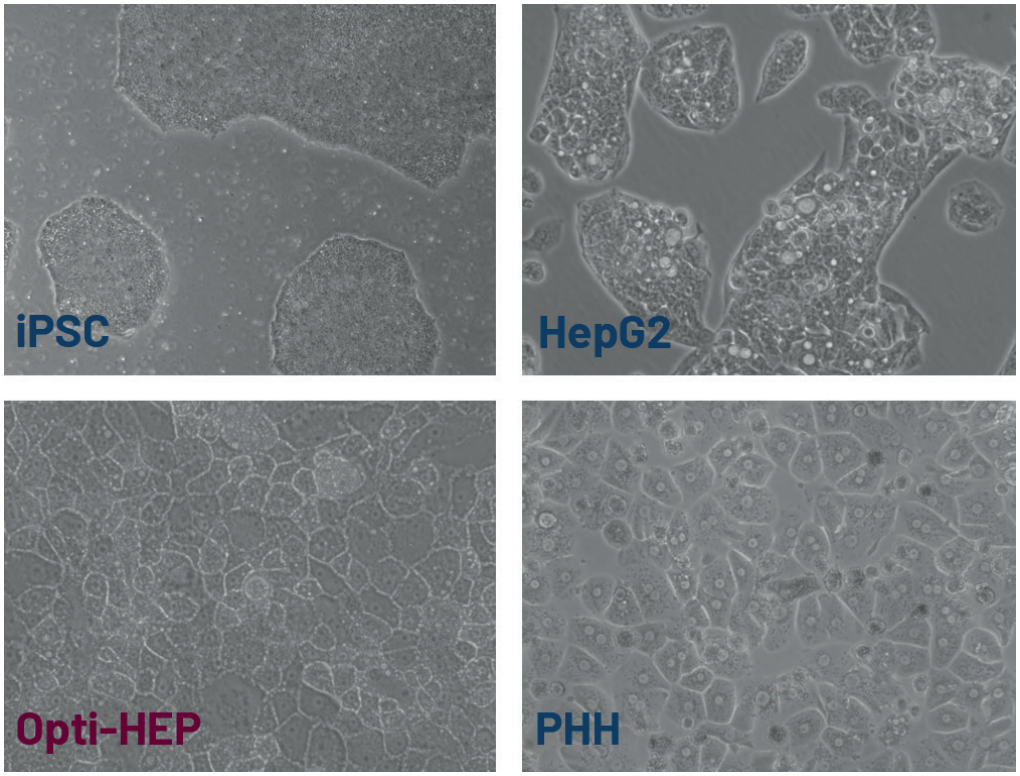


Figure 1: Representative cell morphology pictures of induced pluripotent stem cells (iPSCs), hepatocellular carcinoma HepG2 cells, DefiniGEN Opti-HEP, and primary human hepatocytes (PHH). The pictures reveal the characteristic cobblestone morphology of Opti-HEP, and the presence of a uniform monolayer following >3 weeks of iPSC differentiation. Objective: 20x.

Maturity marker analysis

DefiniGEN Opti-HEP express similar levels of liver maturity markers compared to primary human hepatocytes (PHH).

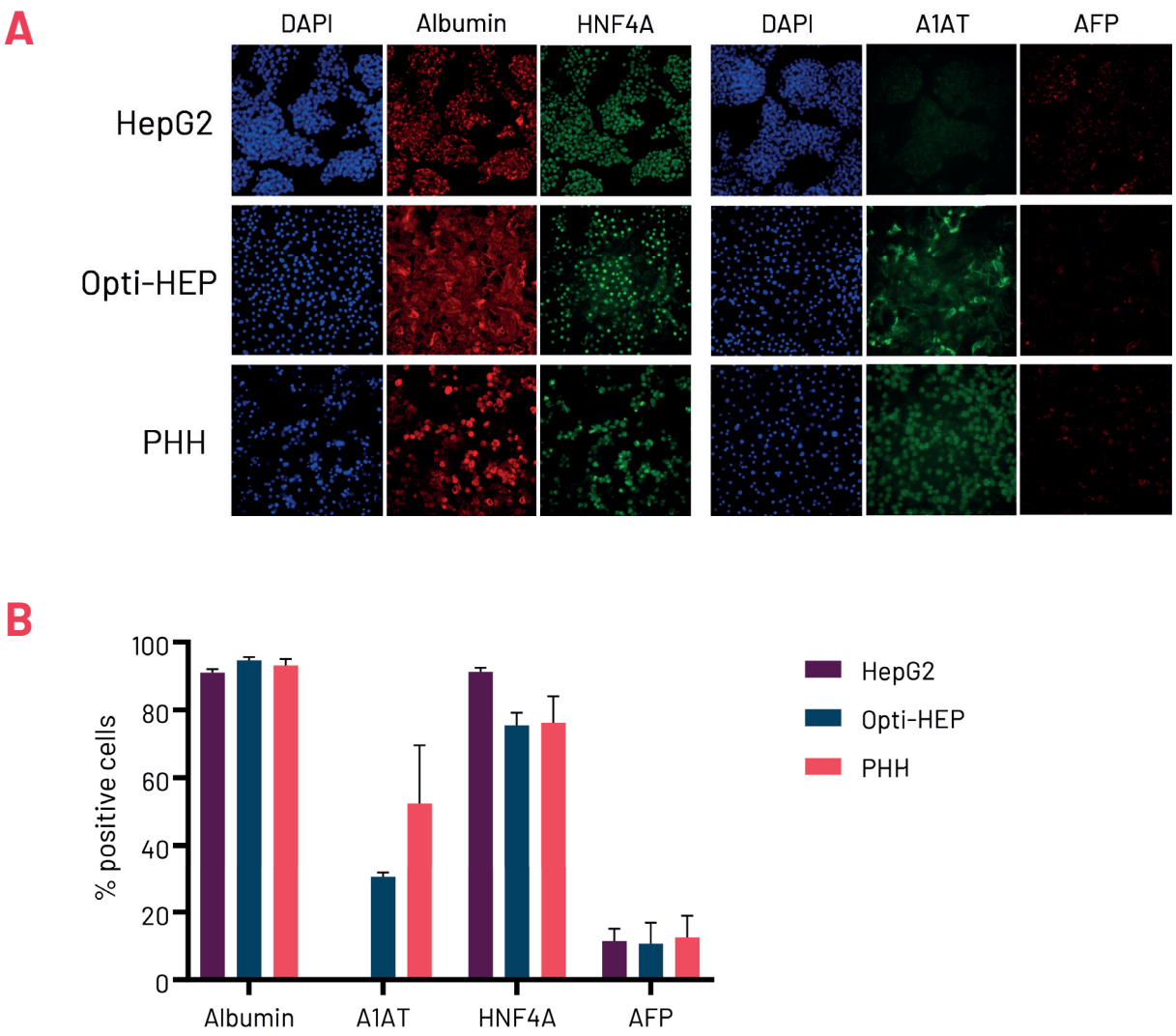


Figure 2: Representative immunocytochemistry pictures and protein quantification showing expression levels of the hepatocyte maturity markers albumin (red), alpha-1-antitrypsin (A1AT; green), HNF4A (green), and AFP (red) in liver carcinoma HepG2 cells, DefiniGEN Opti-HEP, and primary human hepatocytes (PHH; 3 donors). Cells were counterstained with DAPI, and data are presented as mean±SEM of n=3-4 independent experiments.

Urea cycle marker analysis

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

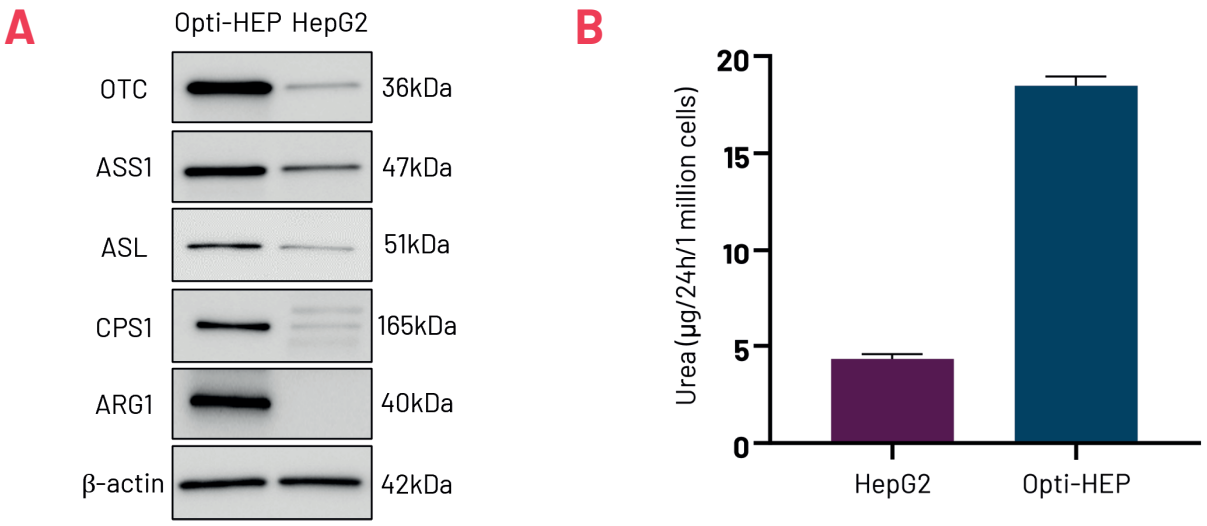


Figure 3: A) Protein expression levels of the urea cycle enzymes OTC, ASS1, ASL, CPS1, and ARG1 in liver carcinoma HepG2 cells and DefiniGEN Opti-HEP. B) Urea secretion in liver carcinoma HepG2 cells and DefiniGEN Opti-HEP. Data are presented as mean±SEM of n=3-4 independent experiments.

Functional gluconeogenesis

DefiniGEN Opti-HEP demonstrate functional gluconeogenesis pathway and respond to gluconeogenesis inducers.

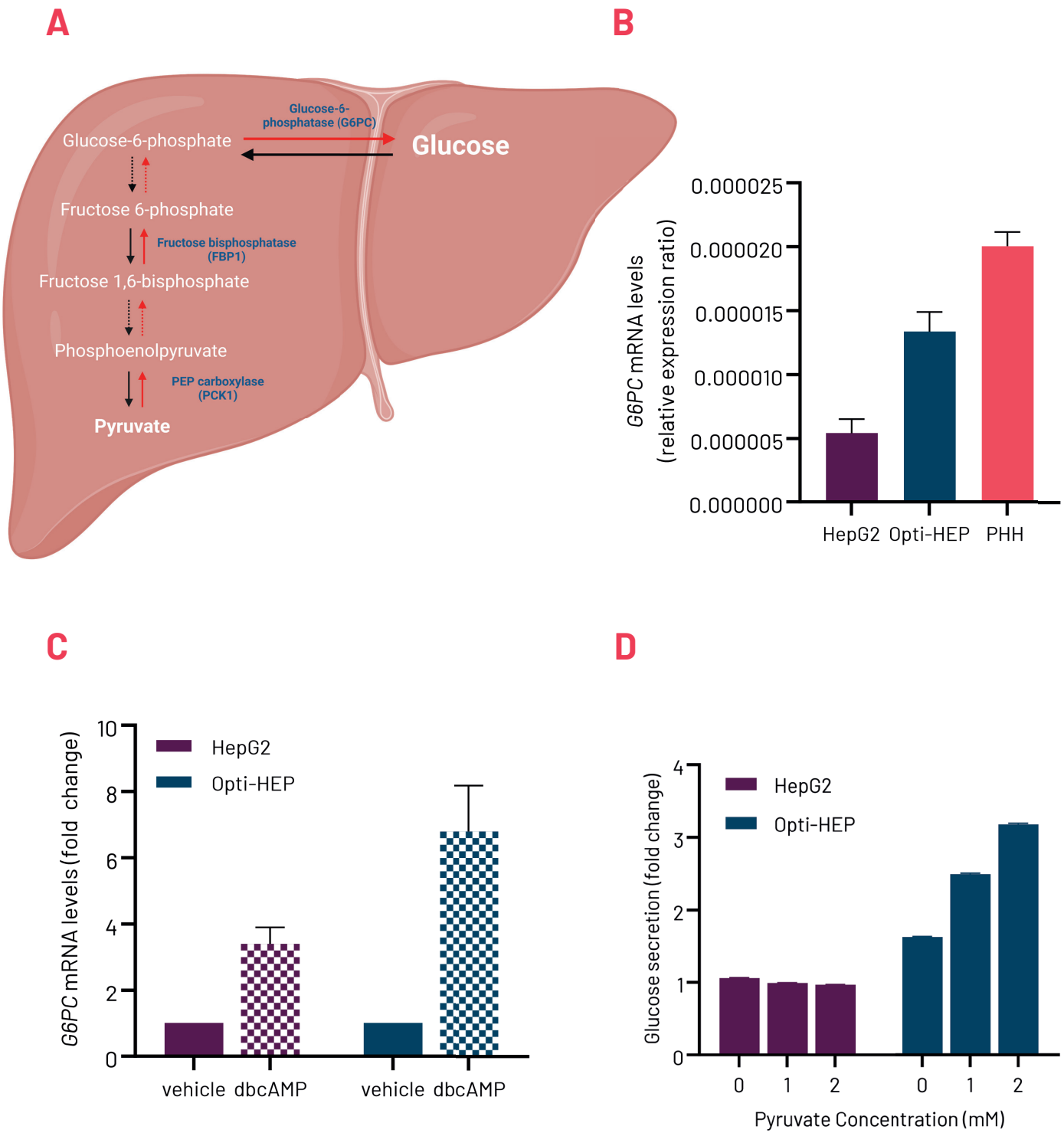


Figure 4: A) Simplified schematic of the gluconeogenesis pathway within human liver. B) G6PC mRNA levels in liver carcinoma HepG2 cells, DefiniGEN Opti-HEP, and primary human hepatocytes (PHH). C) G6PC mRNA levels in liver carcinoma HepG2 cells and DefiniGEN Opti-HEP treated with 0.1mM dbcAMP (gluconeogenesis inducer). D) Glucose secretion in dbcAMP-treated liver carcinoma HepG2 cells and DefiniGEN Opti-HEP upon pyruvate challenge. Data are presented as mean±SEM of n=3-4 independent experiments. mRNA expression data were normalized to 18S rRNA.

CYP450 expression and activity

DefiniGEN Opti-HEP demonstrate comparable levels of CYP450 markers and CYP3A4 activity to primary human hepatocytes.

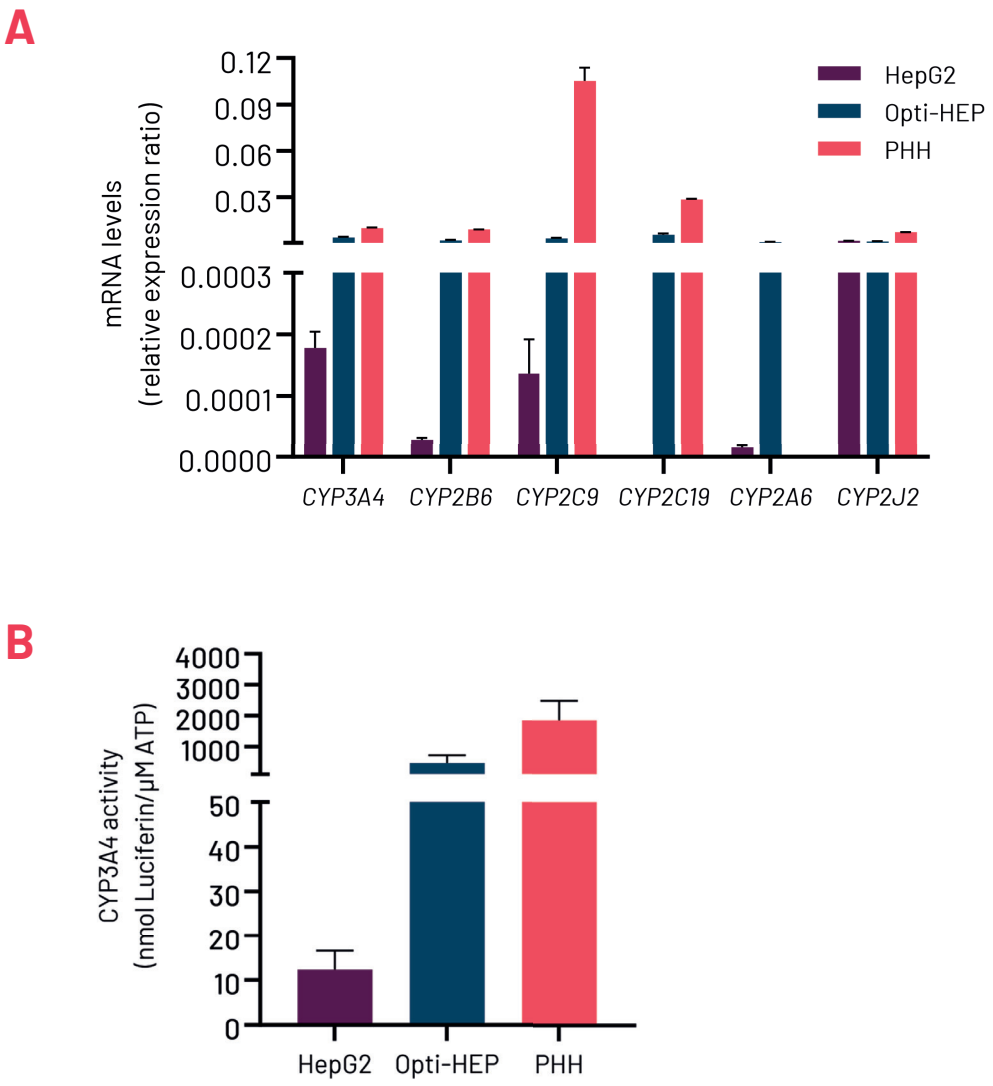
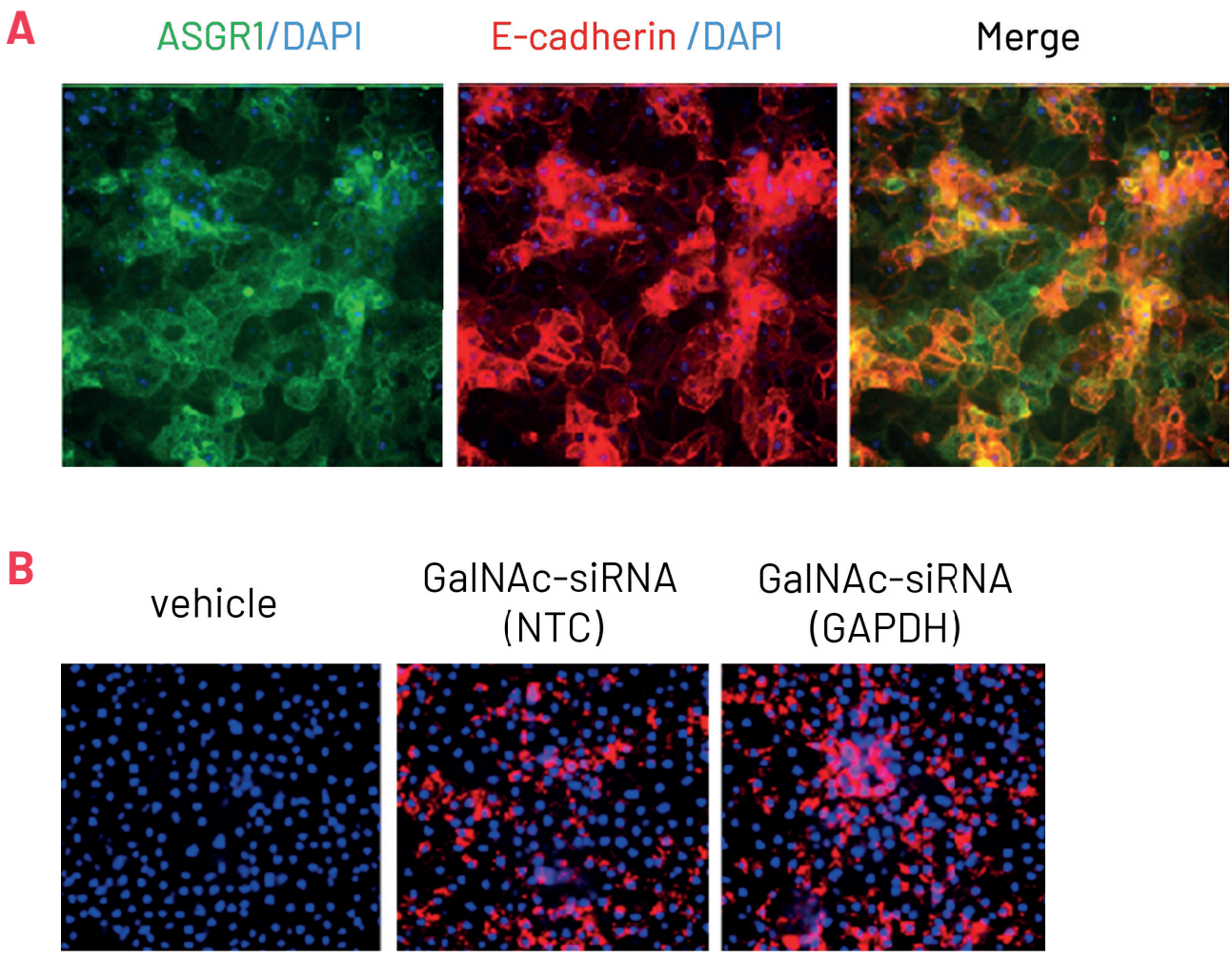


Figure 5: A) mRNA expression levels of Phase I CYP450 genes in liver carcinoma HepG2 cells, DefiniGEN Opti-HEP, and primary human hepatocytes (PHH). B) Basal CYP3A4 activity in liver carcinoma HepG2 cells, DefiniGEN Opti-HEP, and PHH. mRNA data were normalized to the housekeeping gene 18S rRNA and are presented as mean±SEM of n=3-4 independent experiments. CYP3A4 activity data were normalized to ATP levels and are presented as mean±SEM of n=3-5 independent experiments. For PHH data, cells from 3 independent donors were used.

ASGR1 expression and function

DefiniGEN Opti-HEP demonstrate functional membrane localization and activity of the Asialoglycoprotein receptor 1 (ASGR1).



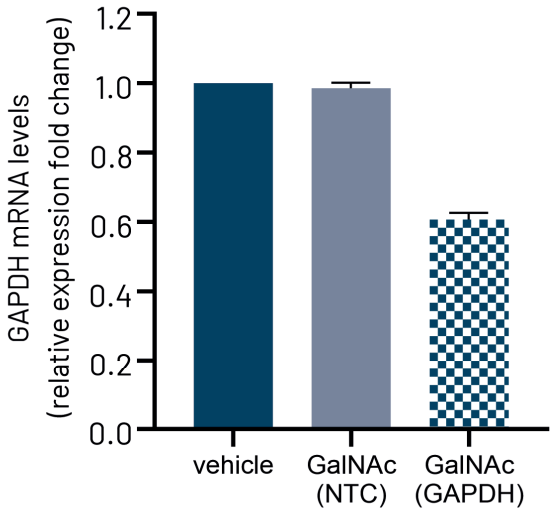


Figure 6: A) Representative immunocytochemistry pictures showing the localization of ASGR1 in the Opti-HEP membrane. Cells were counterstained with the membrane marker E-cadherin and DAPI. B) The effect of ASGR1 in the transport of GalNAc-siRNA conjugate targeting GAPDH in Opti-HEP using GalNAc-Cy3 staining and qPCR. Data are presented as mean±SEM of n=3-4 independent experiments. mRNA expression data were normalized to 18S rRNA. NTC: non-template control.

DefiniGEN Limited
Babraham Research Campus, Babraham
Cambridge, CB22 3AT, United Kingdom

For more information, plase contact us:
+44 (0) 1223 497 106
info@definigen.com

www.definigen.com

DCS-OHF-V1-04/2024