DefiniGEN
DISEASE MODEL INNOVATION

Ulti-HEP Functionality

DefiniGEN's proprietary differentiation protocols permit the large-scale generation of iPSC-derived hepatocytes (Ulti-HEP) with field-leading purity and functionality. Importantly, Ulti-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 a functional** gluconeogenesis pathway
- **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 Ulti-HEP demonstrate the characteristic hepatocyte cobblestone morphology.

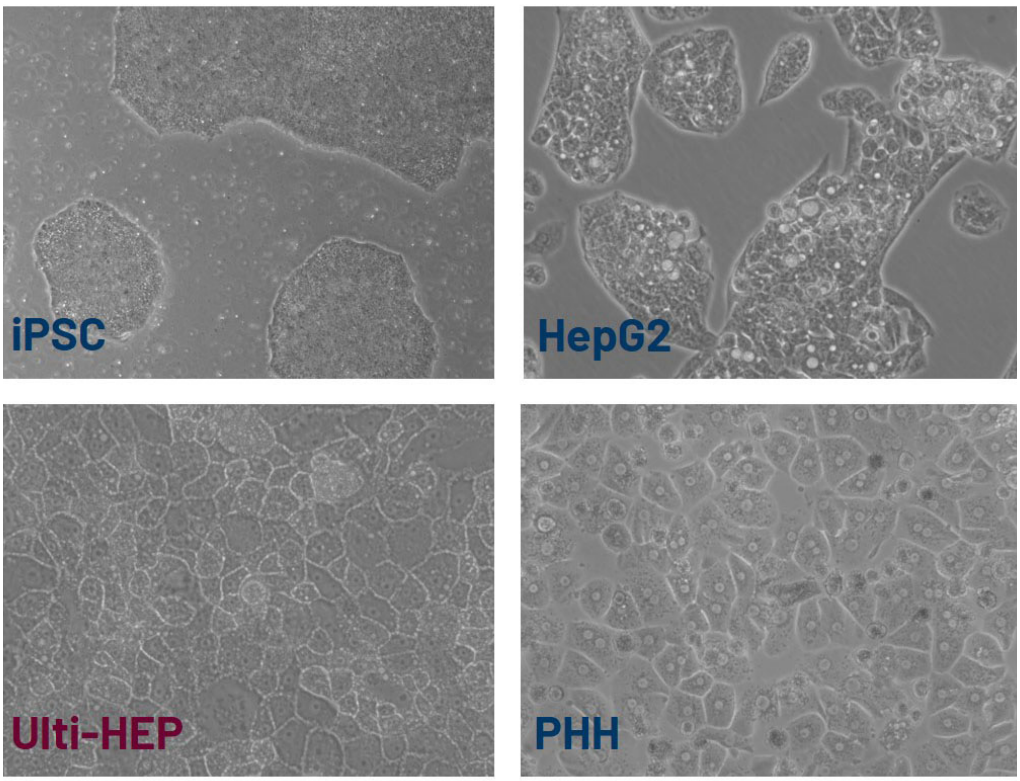

Figure 1: Representative cell morphology pictures of induced pluripotent stem cells (iPSCs), hepatocellular carcinoma HepG2 cells, DefiniGEN Ulti-HEP, and primary human hepatocytes (PHH). The pictures reveal the characteristic cobblestone morphology of Ulti-HEP, and the presence of a uniform monolayer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

Maturity marker analysis

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

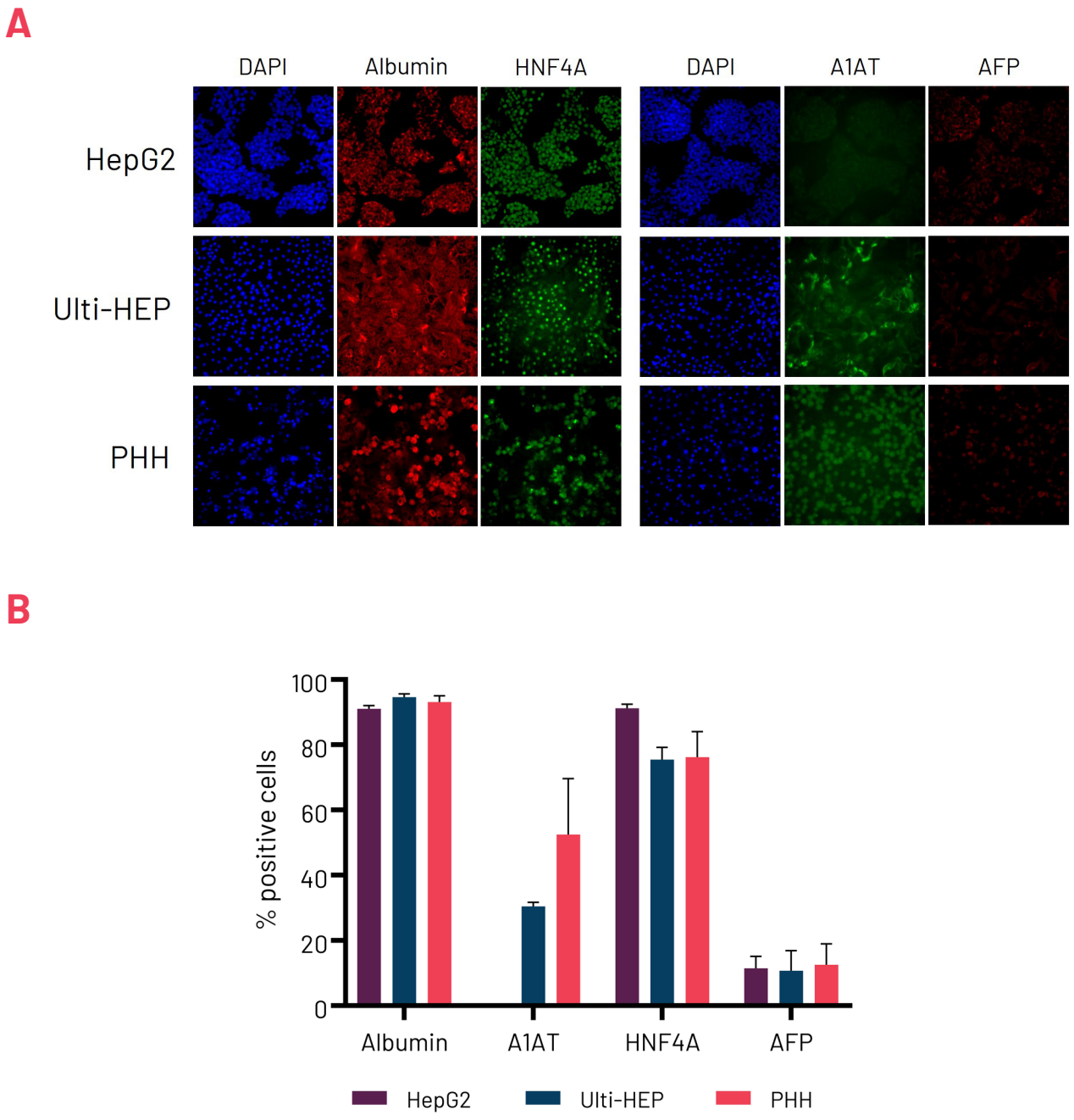

Figure 2: Representative immunocytochemistry pictures and protein quantification showing expression levels of the hepatocyte maturity markers albumin (red), alpha-1-antitrypsin (A1AT; green), HNF4A (green), and AFP (red) in liver carcinoma HepG2 cells, DefiniGEN Ulti-HEP, and primary human hepatocytes (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 Ulti-HEP express higher levels of urea cycle markers and secrete higher levels of urea compared to liver carcinoma cell lines.

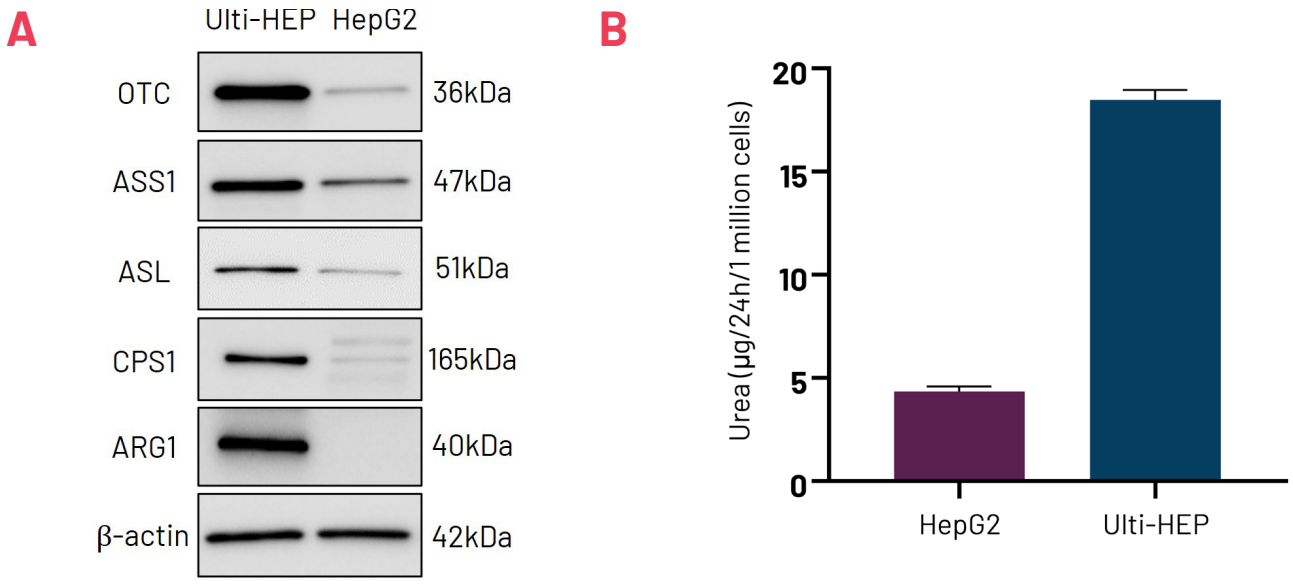

Figure 3: A) Protein expression levels of the urea cycle enzymes OTC, ASS1, ASL, CPS1, and ARG1 in liver carcinoma HepG2 cells and DefiniGEN Ulti-HEP. B) Urea secretion in liver carcinoma HepG2 cells and DefiniGEN Ulti-HEP. Data are presented as mean±SEM of n=3-4 independent experiments.

Functional gluconeogenesis

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

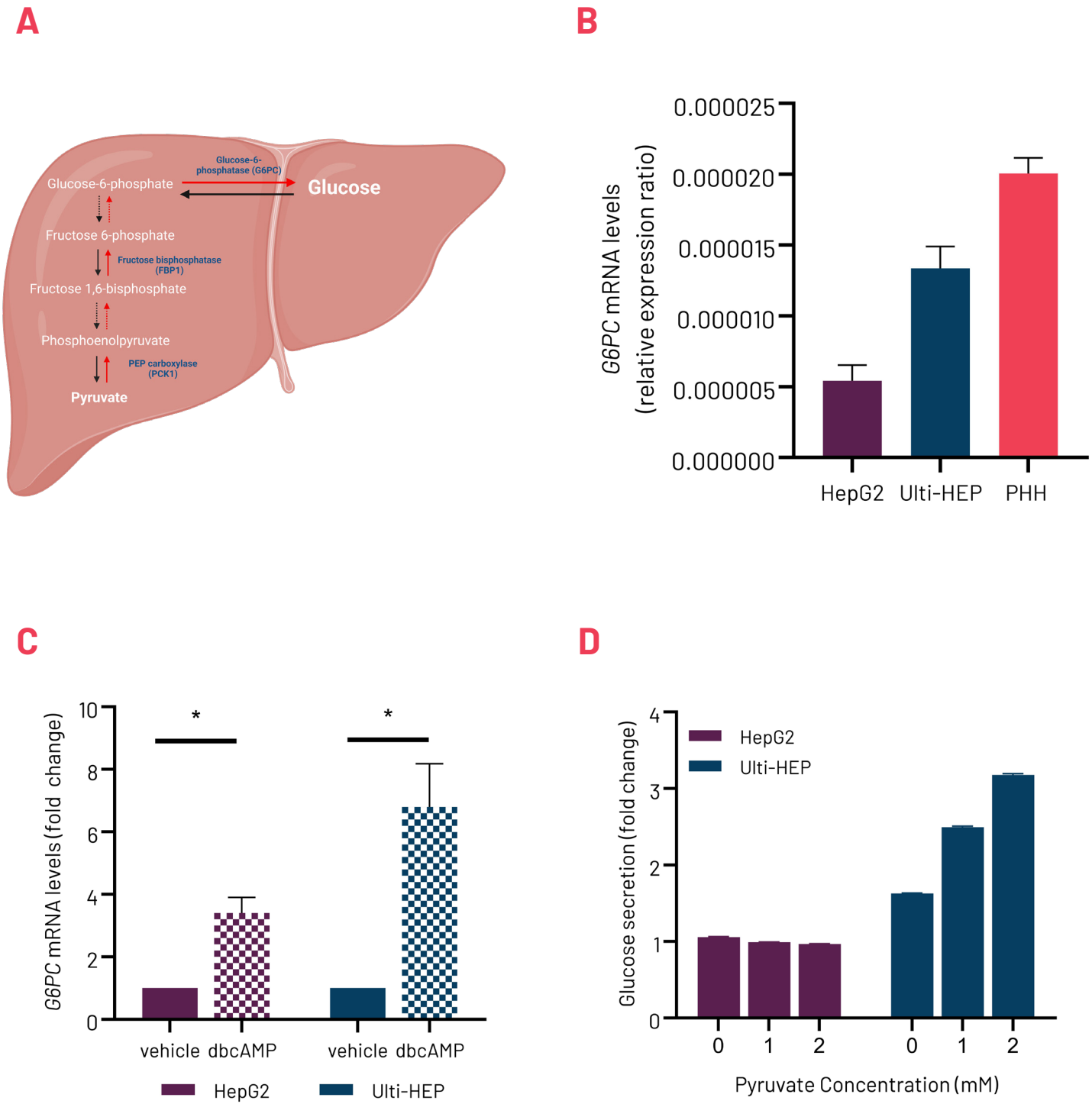

Figure 4: A) Simplified schematic of the gluconeogenesis pathway within human liver. B) G6PC mRNA levels in liver carcinoma HepG2 cells, DefiniGEN Ulti-HEP, and primary human hepatocytes (PHH). C) G6PC mRNA levels in liver carcinoma HepG2 cells and DefiniGEN Ulti-HEP treated with 0.1mM dbcAMP (gluconeogenesis inducer). D) Glucose secretion in dbcAMP-treated liver carcinoma HepG2 cells and DefiniGEN Ulti-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 Ulti-HEP demonstrate comparable levels of CYP450 markers and CYP3A4 activity to primary human hepatocytes.

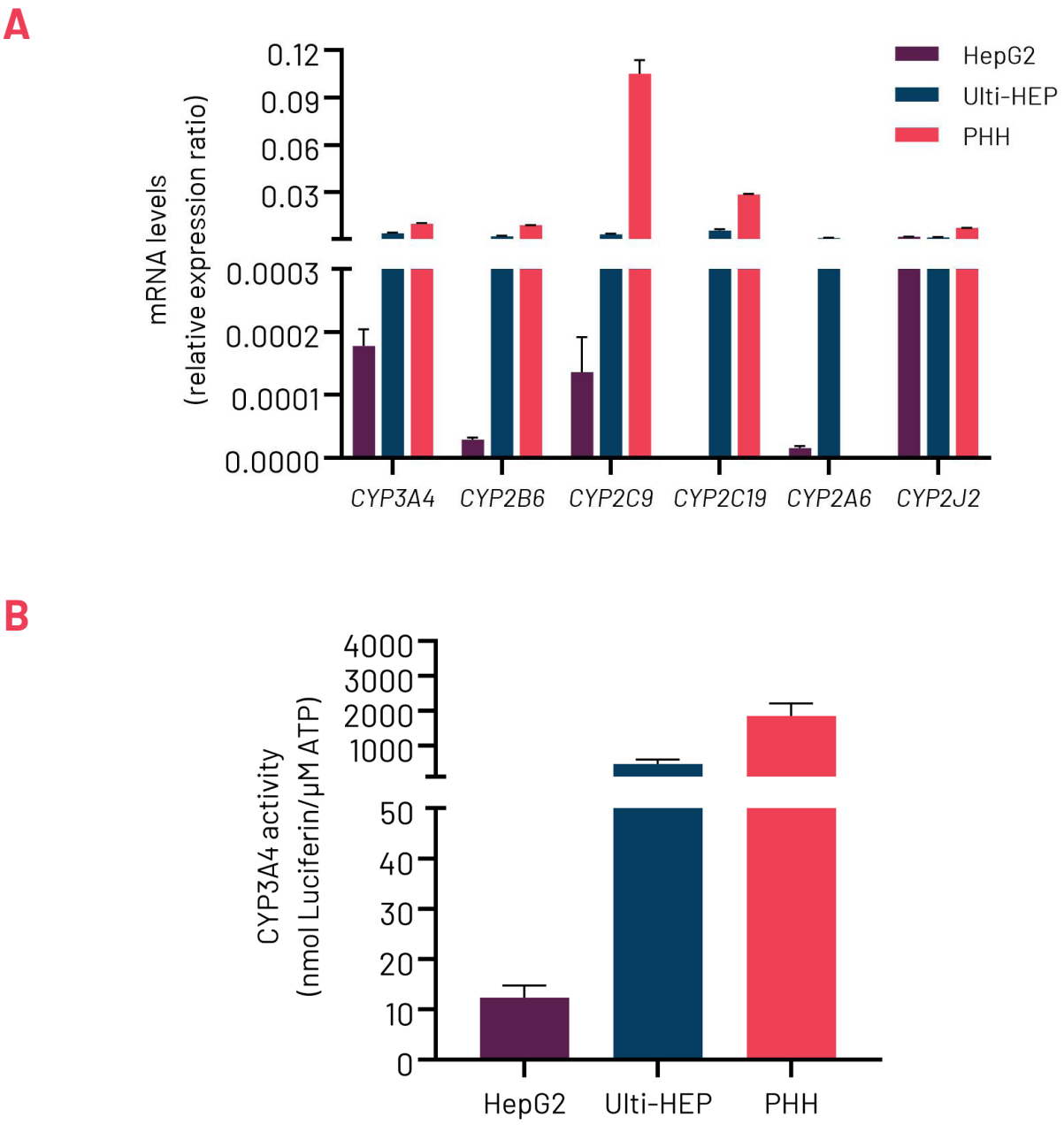

Figure 5: A) mRNA expression levels of Phase I CYP450 genes in liver carcinoma HepG2 cells, DefiniGEN Ulti-HEP, and primary human hepatocytes (PHH). B) Basal CYP3A4 activity in liver carcinoma HepG2 cells, DefiniGEN Ulti-HEP, and PHH. mRNA data were normalized to the housekeeping gene 18S rRNA and are 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 Ulti-HEP demonstrate functional membrane localization and activity of the Asialoglycoprotein receptor 1 (ASGR1).

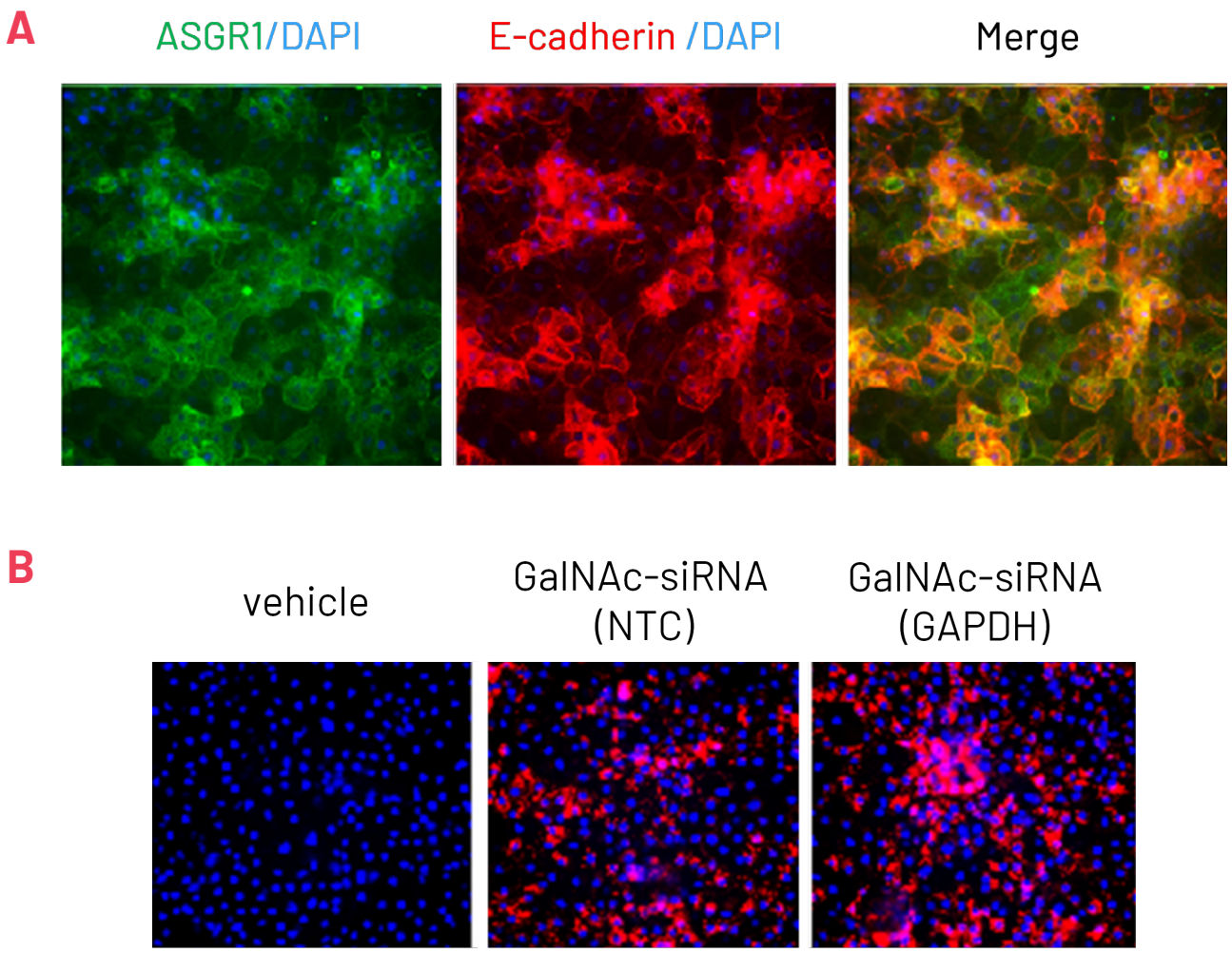

Figure 6: A) Representative immunocytochemistry pictures showing the localization of ASGR1 in the Ulti-HEP membrane. Cells were counterstained with the membrane marker E-cadherin and DAPI. B) The effect of ASGR1 in the transport of GalNAc-siRNA conjugate targeting GAPDH in Ulti-HEP using GalNAc-Cy3 staining 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.

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