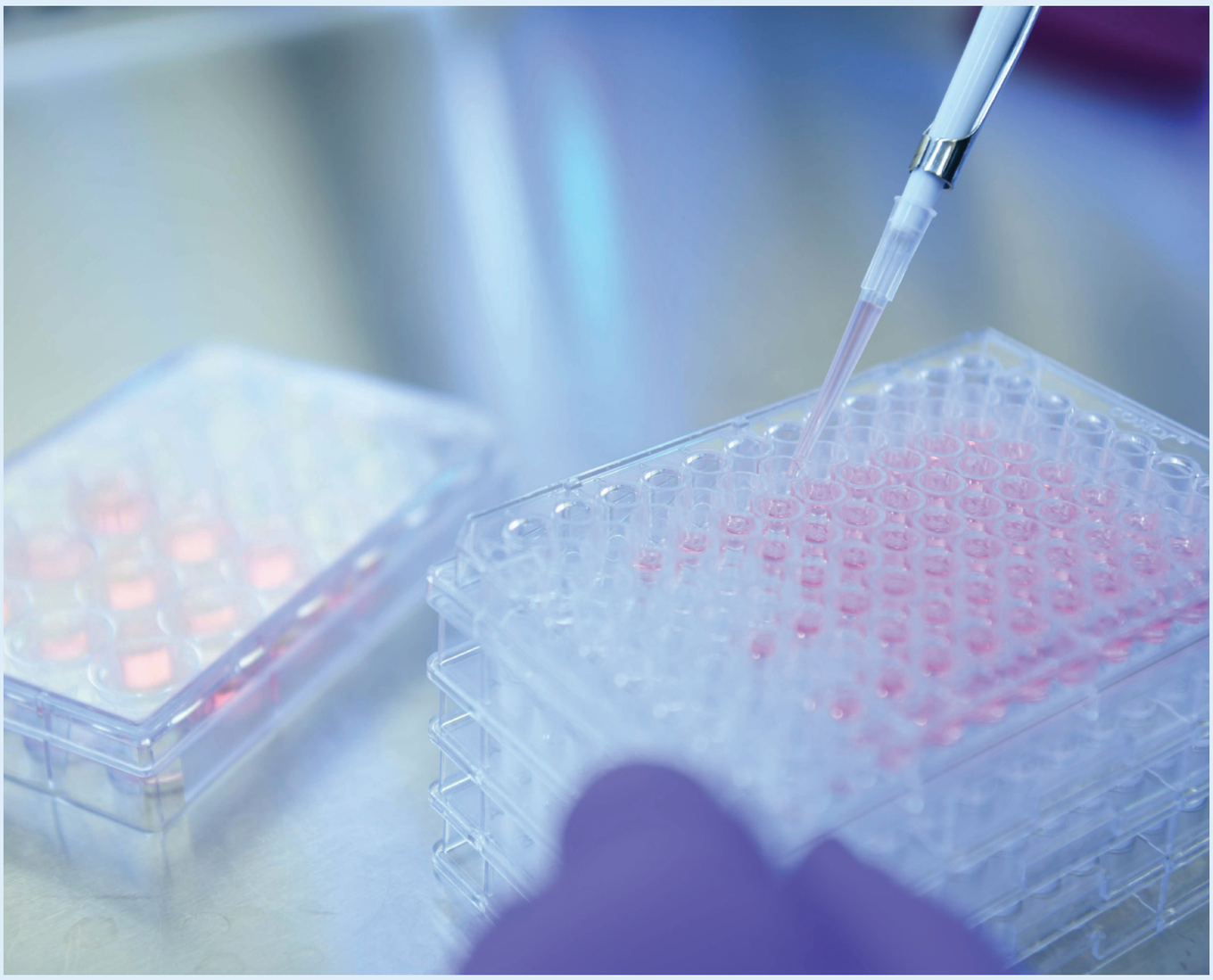
DefiniGEN
DISEASE MODEL INNOVATION

**Toxicology Screening**

# DefiniGEN Ulti-HEP offer an improved cellular platform for *in vitro* ADME and toxicology screening compared to hepatocellular carcinoma cell lines.

In the early stages of drug discovery, *in vitro* models play a crucial role in generating clinically relevant data, expediting the progress of potential therapeutics into clinical trials. However, despite the careful approaches taken during drug development, most therapeutics still fail to reach clinical trials due to the lack of translatability between pre-clinical models and the clinic.

Primary human hepatocytes (PHH) and hepatocellular cancer cells are the current pre-clinical *in vitro* models used. However, they come with limitations. DefiniGEN's highly characterised human induced pluripotent stem cell (iPSC) derived hepatocytes (Ulti-HEP) overcome these limitations, now offering a sustainable *in vitro* platform for ADME and toxicology screening.



## Ulti HEP Functionality

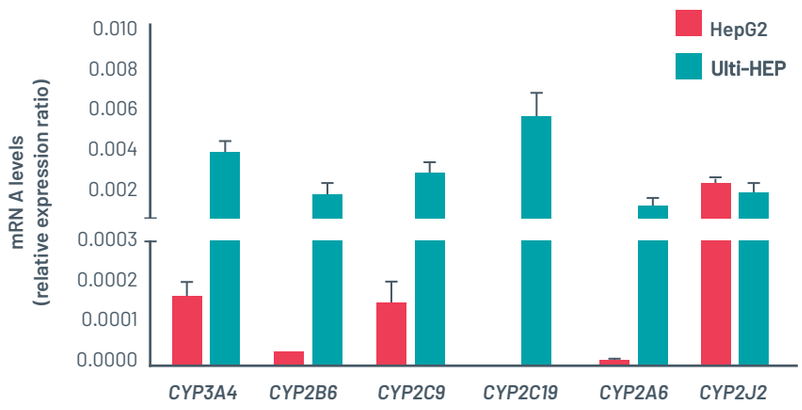
### Cytochrome P450 expression and activity

The cytochrome P450 (CYP) enzymes are the major players in drug metabolism, being responsible for the metabolism of >90% of the drugs that are currently used in clinic. CYPs are mainly expressed in the liver and catalyze the Phase I reactions, whereupon drugs can be oxidized, reduced, or hydrolyzed, downstream leading to the generation of products with either decreased or increased toxicity. To date, 57 enzymes have been discovered, from which the isoforms belonging to the CYP1, CYP2, and CYP3 families contribute to the metabolism of approx. 80% of clinical drugs. Primary human hepatocytes are considered the gold standard *in vitro* model in ADME and toxicology screening. But, they come with limitations, including short-life span, rapid loss of function, and limited supply. Due to these limitations, hepatocellular carcinoma cell lines, including HepG2 and HepaRG, are used instead. However, the malignant origin of these models, in addition to the lack of drug metabolizing enzyme expression they demonstrate hinders data interpretation.

DefiniGEN's improved iPSC differentiation protocols now lead to the generation of Ulti-HEP with functional CYP450 enzyme expression and activity. In Figure 1, we demonstrate that Ulti-HEP express significantly higher mRNA levels of various CYP enzymes compared to HepG2 carcinoma cells, including *CYP3A4, CYP2B6, CYP2C9, CYP2C19*, and *CYP2A6*. Importantly, we show the activity levels of CYP3A4 in DefiniGEN Ulti-HEP, revealing comparable functionality to that seen in primary human hepatocytes and significantly higher to that of HepG2 cells.

**A**

**Phase I CYP450 genes**




**B**

**Basal CYP3A4 activity**

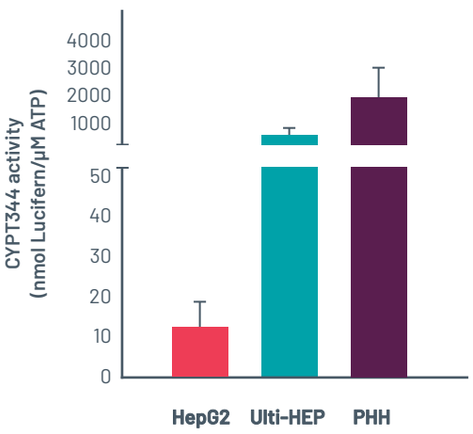



**Figure 1: A)** mRNA expression levels of Phase I CYP450 genes in liver carcinoma HepG2 cells and DefiniGEN Ulti-HEP. **B)** Basal CYP3A4 activity in liver carcinoma HepG2 cells, DefiniGEN Ulti-HEP, and primary human hepatocytes (PHH). mRNA data were normalized to the housekeeping gene *18SrRNA* and 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.

### Cytochrome P450 induction and inhibition

Induction and inhibition of CYP450 enzymes are central mechanisms in xenobiotic metabolism, resulting in pharmacokinetic drug-drug interactions (DDI). Both mechanisms are of particular clinical importance for therapeutic and toxicological reasons, as inhibition of drug metabolism can lead to undesirable elevations in plasma drug concentrations, whilst CYP induction following prolonged drug treatment has been associated with adverse DDI. Thus, access to *in vitro* models that can accurately predict both mechanisms is crucial in drug therapy.

Here, we show that, unlike HepG2 cells,CYP450 activity can be both induced and inhibited in DefiniGEN Ulti-HEP at comparable levels to those seen in primary human hepatocytes following treatment with known inducers and inhibitors. As proof-of-concept, we demonstrate CYP3A4 activity in HepG2, Ulti-HEP, and primary human hepatocytes following inhibition with the CYP3A4 inhibitor ketoconazole and induction with increasing concentrations of the CYP3A4 inducer 1a,25-hydroxy-vitamin D3.

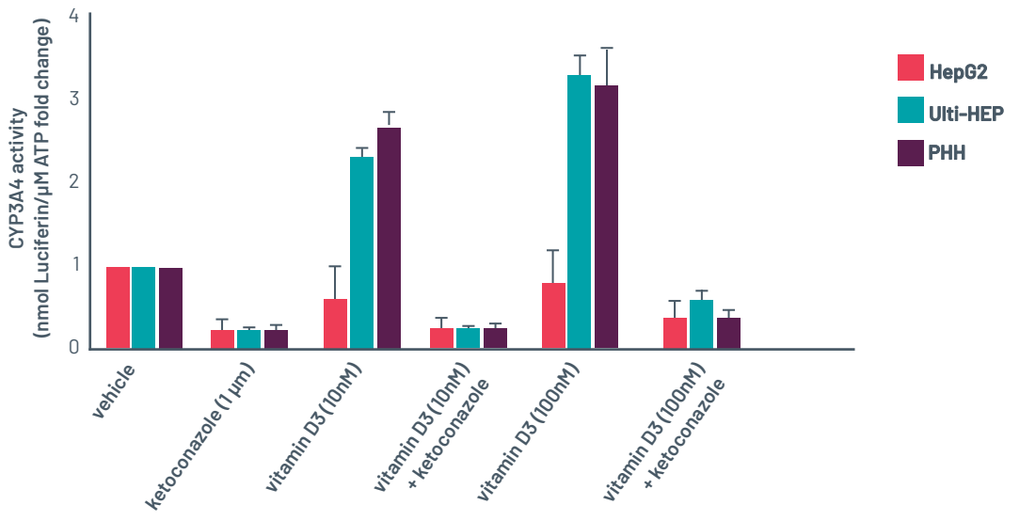



**Figure 2:** CYP3A4 induction and inhibition in liver carcinoma HepG2 cells, DefiniGEN Ulti-HEP and primary human hepatocytes (PHH), following 72h of treatment with vehicle, 1µM ketoconazole (CYP3A4 inhibitor), 10-100nM vitamin D3 (CYP3A4 inducer), or a combination of vitamin D3 and ketoconazole. CYP3A4 activity data were normalized to ATP levels and are presented as mean±SEM fold change of n=3-5 independent experiments. For PHH data, cells from 3 independent donors were used.

## Predicting Toxicity

### Drug-Induced Liver Injury (DILI) predictivity

Drug-induced Liver Injury (DILI) is a leading cause of drug failure in the clinic, accounting for more than 50% of liver failure cases. DILI is mainly the result of poor suitability of the available pre-clinical models to predict toxicity of positive compounds, either due to species-specific differences (e.g., animal models, animal-derived cell lines), donor-to-donor variability and short-life span (e.g., primary human hepatocytes), or low metabolic capacity (e.g., hepatocellular carcinoma cell lines). Recently, efforts to address the short-life span of primary human hepatocytes have resulted in the generation of 3D *in vitro* liver models with promising results. However, these models still fail to address the donor-to-donor variability issue, limiting their suitability for high-throughput screening during the early phases of the discovery process.

Here, we assessed Ulti-HEP ability to accurately predict toxicity risk for a broad set of compounds with known DILI liability. The results revealed that Ulti-HEP can accurately predict DILI across a dose-response using cell viability (ATP content) as the endpoint, consistent with the functional CYP450 enzyme expression and activity demonstrated above. The data sets alongside the expansion capacity and lack of donor-to-donor variability iPSC-derived cell models offer, highlight both the superiority of Ulti-HEP over the current hepatocyte models and their suitability in high-throughput hepatotoxicity screening.

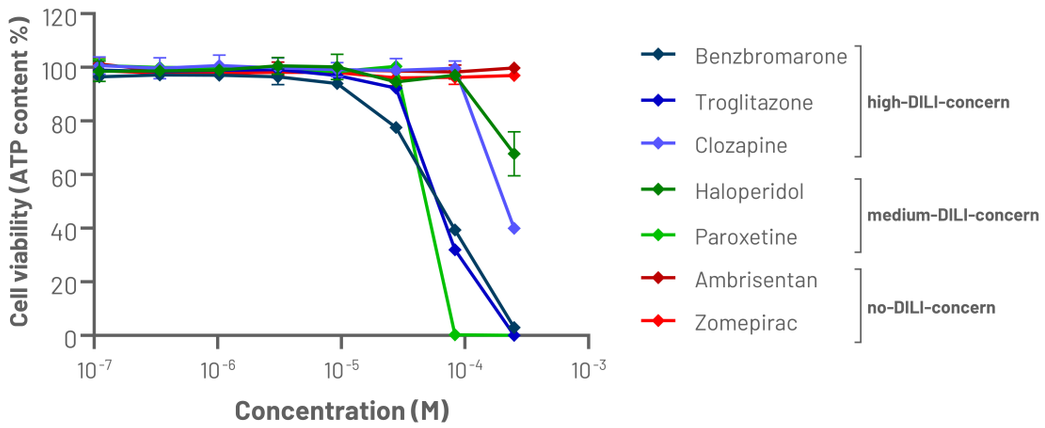



**Figure 3:** Cell viability (ATP content) in DefiniGEN Ulti-HEP following 48h of treatment with increasing concentrations (0-250 µM) of compounds with known DILI liability (classification based on Proctor *et al.* 2017). Cell viability data were normalized to positive (250 µM chlorpromazine) and negative controls (0.2% DMSO) and are presented as mean±SD of n=3 technical replicates.

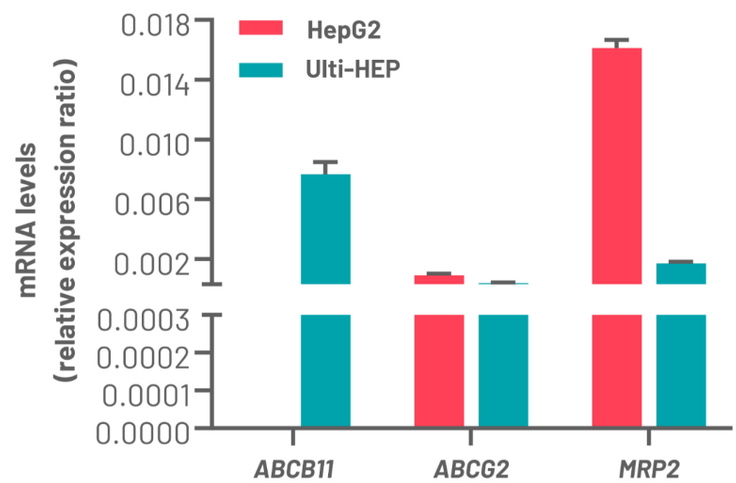
### Efflux transporter expression and cellular localisation

In addition to the central role of CYP450 enzymes in drug metabolism, inhibition of liver-specific efflux transporters, including the bile acid export pump (BSEP/ABCB11) and the multidrug resistance (MDR) proteins (e.g., ABCG2, ABCC2/MRP2) has emerged as a DILI risk factor. Supporting this, *in vitro* screening for BSEP inhibition in drug discovery is now recommended by the European Medicines Agency on the Investigation of Drug Interactions (2012). Despite the need of physiological hepatic cell systems for the accurate *in vitro* **screening of efflux transporter inhibition, the predominant** *in vitro* models that are currently used include non-hepatic cell lines (e.g., HEK293) that will only artificially **over-express** a single transporter. This is because transporter expression in hepatocellular carcinoma cells is either absent or lacks proper cellular **localization**.

Here, we show that DefiniGEN's Ulti-HEP express functional levels of the efflux transporters required (e.g., *ABCB11, ABCG2, MRP2)* for large-scale toxicity screening. Crucially, we demonstrate the cellular **localization** of these transporters in the hepatocyte membrane, confirming their functional role in bile acid and drug transport.

## A

### Efflux transporters



B

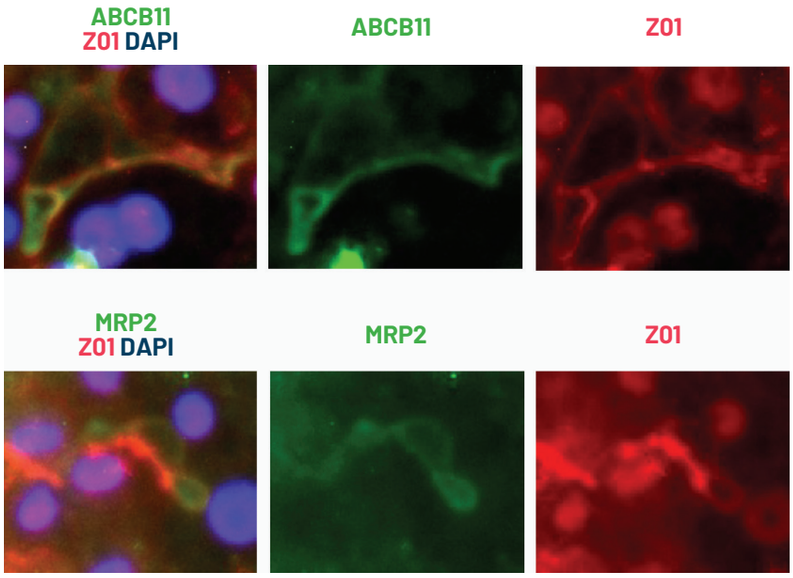
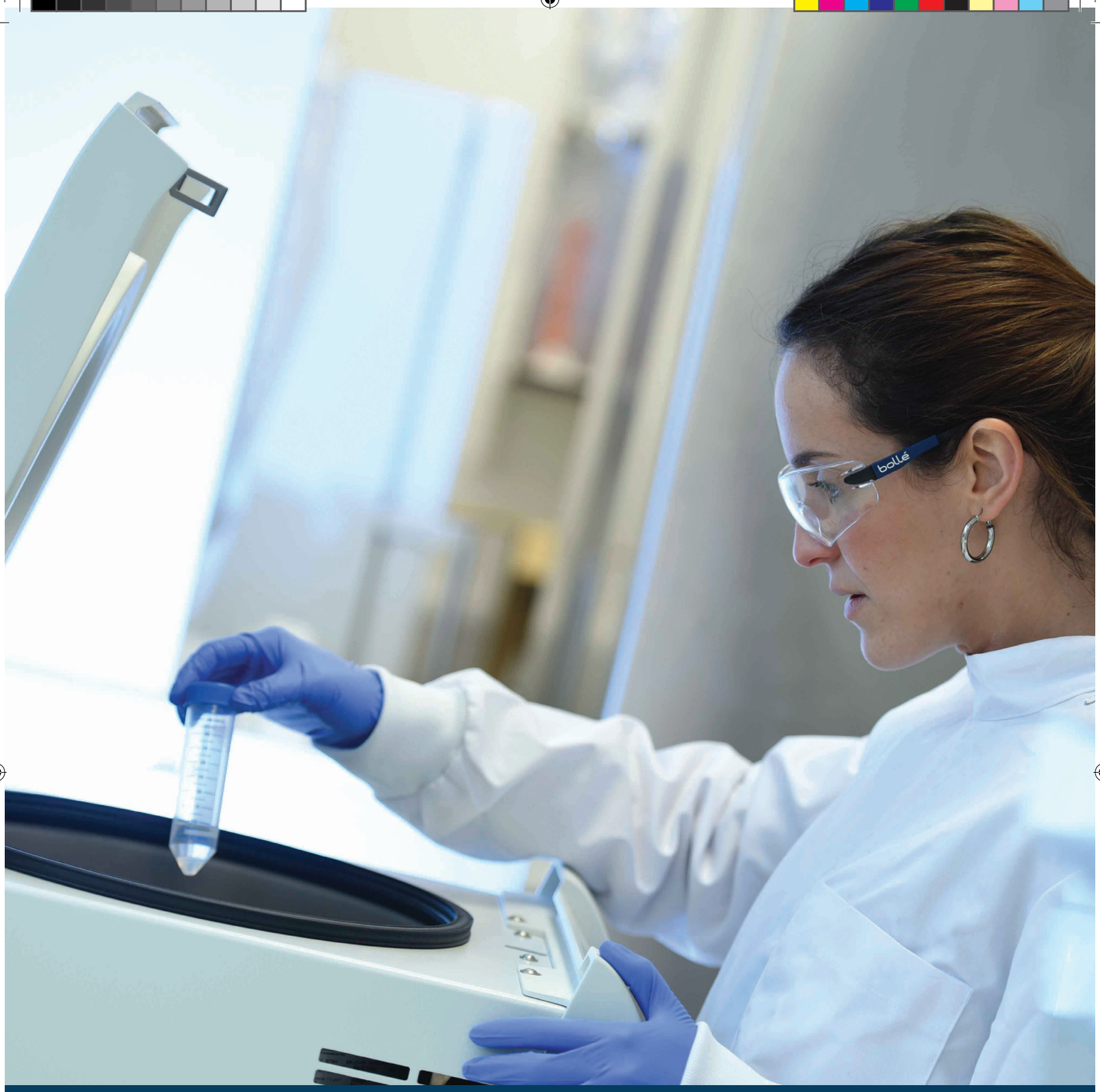



**Figure 4: A)** mRNA expression levels of efflux transporter genes *ABCB11, ABCG2*, and *MRP2* in liver carcinoma HepG2 cells and DefiniGEN Ulti-HEP. mRNA data were normalised to the housekeeping gene *18SrRNA* and are presented as mean±SEM of n=3-4 independent experiments. **B)** Protein expression of efflux transporter proteins ABCB11 and MRP2 (green), co-stained with the apical marker ZO1 (red) in sandwich-cultured Ulti-HEP by immunocytochemistry (ICC). Nuclei counterstaining with DAPI (blue).

It should be noted that, in addition to the endpoint assays shown above, our cells can be used for the measurement of additional endpoint clinical biomarkers that are routinely tested during DILI screening, including albumin, urea, and ALT (data not shown).

DefiniGEN Ulti-HEP offer a superior *in vitro* liver platform for high-throughput ADME and toxicology screening, thereby minimizing risks while reducing costs, and paving the way for a more efficient and effective future in the field of drug discovery. Please contact our scientists for additional information at info@definigen.com



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