

Functionally Relevant hPSC-Derived Hepatocytes and Liver Organoids for Hepatotoxicity and Liver Biology Modeling

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INTRODUCTION

Functionally relevant human hepatocyte models are critical for drug safety and efficacy screening, cell therapy, and the study of liver biology and diseases. Conventional systems for liver studies present challenges, including limited human relevance of rodent models, rapid de-differentiation of primary human hepatocytes (PHHs) in culture, and a lack of metabolic maturity in immortalized cell lines such as HepG2 and Huh7. To address these challenges, we developed specialized media to generate functionally relevant human pluripotent stem cell (hPSC)-derived hepatocyte models. STEMdiff™ Hepatocyte Kit is a three-stage serum-free differentiation protocol that supports efficient and reproducible generation of hepatocyte-like cells (HLCs) over 21 days. Human induced pluripotent (hiPSC) or human embryonic (hESC) stem cells are patterned to definitive endoderm (DE) cells, followed by specification to hepatic progenitor (HP) cells and finally, maturation to HLCs. Both HP cells and HLCs can be used to establish hPSC-derived liver organoids using STEMdiff™ Hepatic Organoid Growth Medium. These expandable organoids are compatible with long-term expansion, cryopreservation, and further maturation using STEMdiff™ Hepatic Organoid Differentiation Medium. HLCs and further differentiated hPSC-derived liver organoids exhibit mature hepatic functions and sensitivity to known hepatotoxic compounds, demonstrating the utility of the STEMdiff™ Hepatocyte Kit and STEMdiff™ Hepatic Organoid Media workflows in liver modeling and drug screening applications.

METHODS

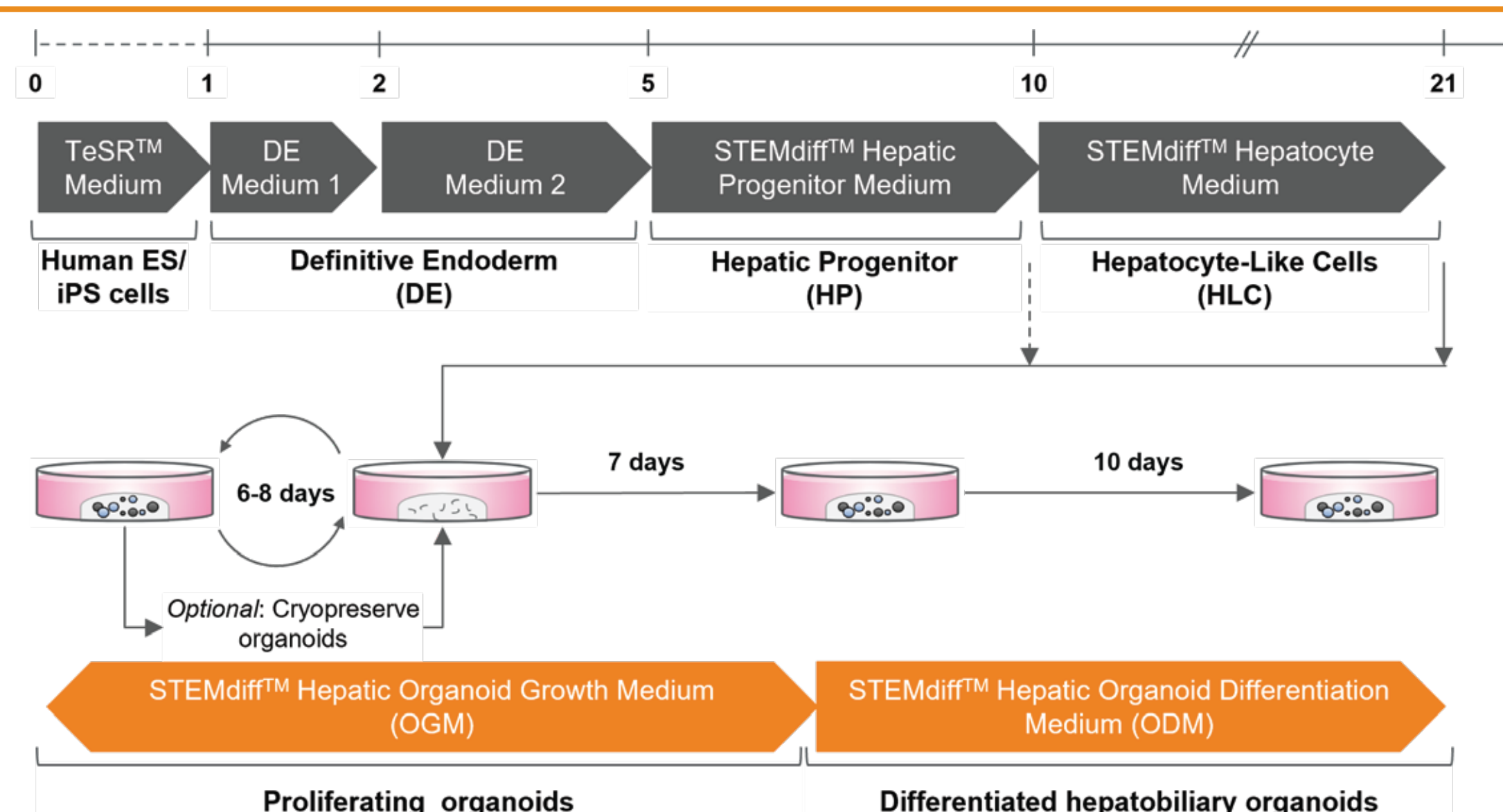


FIGURE 1. Protocols for Generating hPSC-Derived Hepatocyte-Like Cells and Liver Organoids

On day 0, single-cell suspensions of hPSCs maintained in mTeSR™1, mTeSR™ Plus, eTeSR™, or TeSR™-AOF were seeded onto Laminin-521-coated 6-, 24-, or 96-well tissue culture plates. Cells were maintained in the appropriate TeSR™ medium supplemented with 10 μM Y-27632 for the first 24 hours. Differentiation was initiated on day 1 by performing a full-medium change using STEMdiff™ Definitive Endoderm (DE) Medium 1, followed by three full-medium changes using STEMdiff™ DE Medium 2 on days 2, 3, and 4. On days 5, 6, 7, and 9, full-medium changes were performed using STEMdiff™ Hepatic Progenitor Medium. For the last 11 days of the differentiation, cells were cultured in STEMdiff™ Hepatocyte Medium, with full-medium changes performed every 2 days. To generate proliferative hPSC-derived liver organoids, HP and HLC monolayer cultures were mechanically dissociated on day 10 or 21 respectively, replated into Corning® Matrigel® domes, and maintained in STEMdiff™ Hepatic Organoid Growth Medium (OGM). These organoids were serially passaged as fragments in OGM every 6-8 days, and medium changes were performed 2-3 times per week. To further differentiate the organoids, organoid fragments were seeded in OGM, and after 7 days of expansion, cultures were maintained in STEMdiff™ Hepatic Organoid Differentiation Medium (ODM) for 10 days, with full-medium changes performed every 3 days.

RESULTS

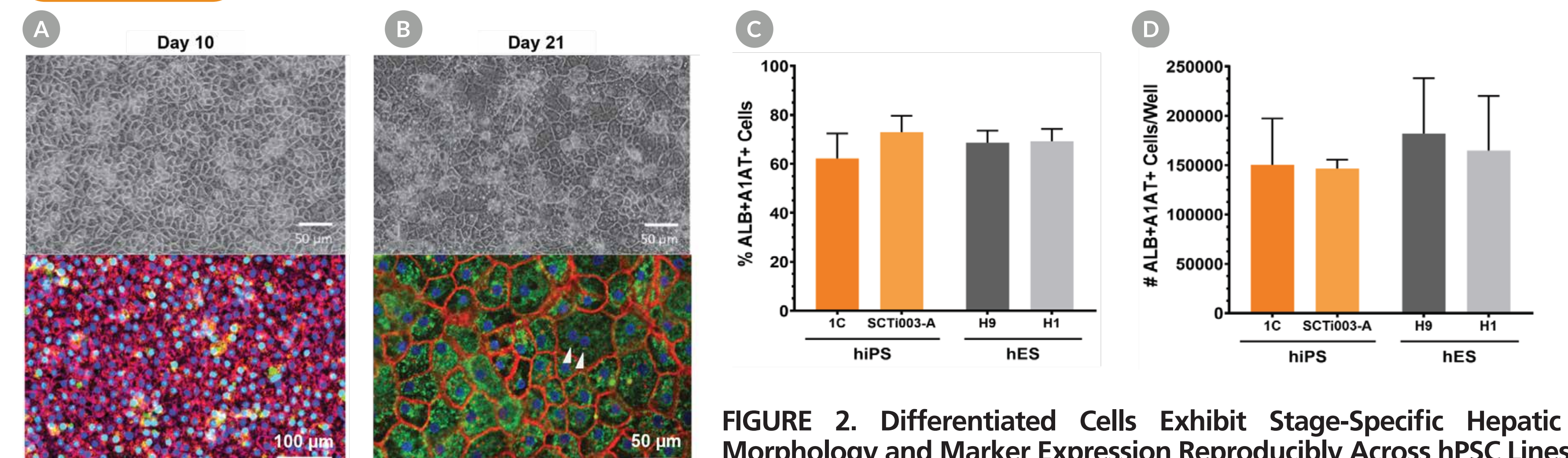


FIGURE 2. Differentiated Cells Exhibit Stage-Specific Hepatic Morphology and Marker Expression Reproducibly Across hPSC Lines

(A) On day 10 of differentiation, cells exhibited polygonal morphology and uniform expression of fetal hepatocyte marker AFP (red), hepatic transcription factor HNF6 (green), and ductal marker CK19 (magenta), consistent with a bi-phenotypic hepatic progenitor identity. (B) By day 21, decreased nucleus-to-cytoplasm ratios were observed, along with uniform expression of mature hepatocyte marker ALB (green), and tight junction marker EPCAM (red), as well as instances of binucleation (white arrowheads), all characteristic features of hepatocytes. HLCs generated using two hiPSC and hESC lines each were analyzed by flow cytometry on day 21. On average, (C) 66 ± 8.1% of cells, or (D) 1.65 × 10⁵ cells per well of a 24-well plate expressed mature hepatocyte markers ALB and A1AT (mean ± SD, n = 2 - 9).

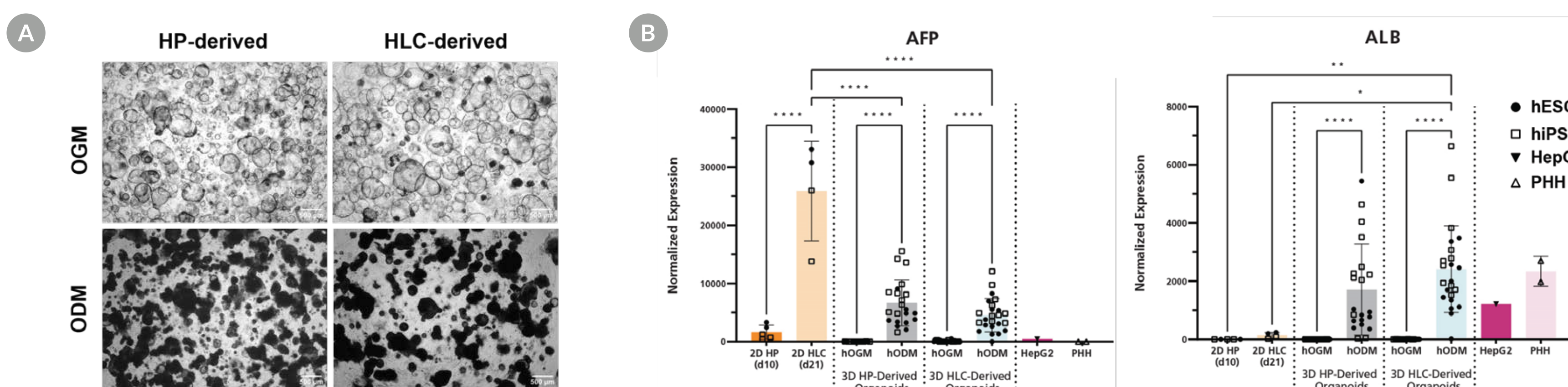


FIGURE 3. hPSC-Derived Liver Organoids Undergo a Change in Morphology and Exhibit Gene Expression Consistent with Hepatic Maturation when Further Differentiated

(A) Proliferating hPSC-derived liver organoids derived from HPs or HLCs distinctly changed morphology when differentiated further using ODM, resulting in a compact and dense phenotype that was often accompanied by thickened epithelia (passage 9 shown, representative of passages 1 - 15). (B) Gene expression levels for organoids expanded and differentiated across 10 passages were characterized by qPCR and normalized to the housekeeping gene *TBP*. Fetal hepatocyte marker *AFP* was significantly downregulated, and mature hepatic marker *ALB* was significantly upregulated in the HLC-derived differentiated organoids (hODM) as compared to 2D HLCs (mean ± SD, n = 2 experiments). Statistical significance was determined using ordinary one-way ANOVA. ****p < 0.0001, **p = 0.0035, *p = 0.0397.

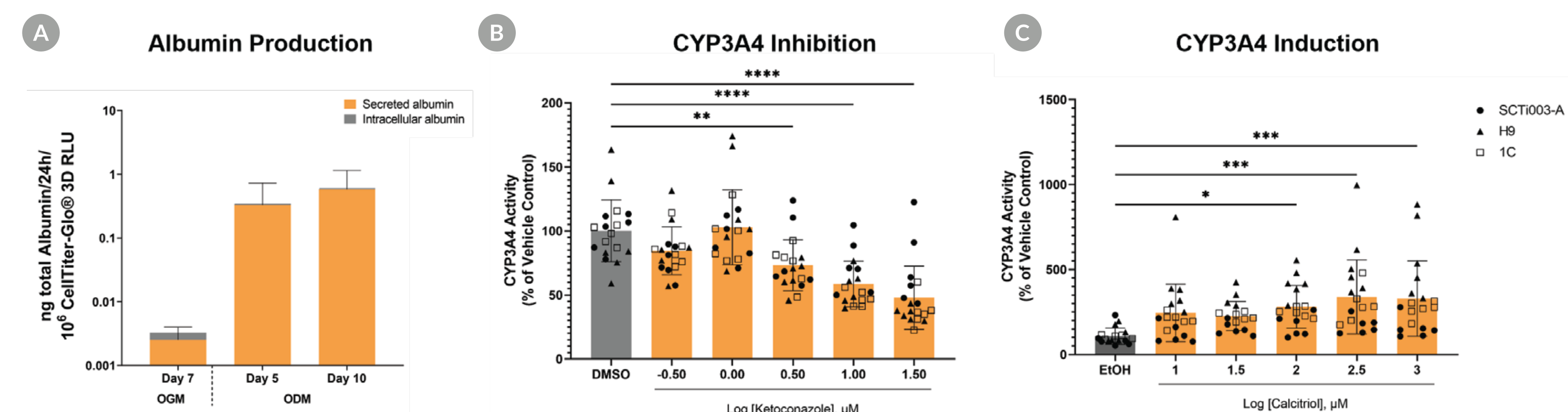


FIGURE 5. Differentiated HLC-Derived Liver Organoids Exhibit Mature Hepatic Functionality That Can Be Modulated by Specific Inducers and Inhibitors

(A) Secreted and intracellular albumin levels were measured in cell culture supernatants and lysates respectively, collected at different time points during HLC-derived organoid differentiation using ODM. The amount of total albumin produced (secreted plus intracellular) increased in a time-dependent manner and was highest at the end of the differentiation (Day 10 in ODM). Differentiated HLC-derived liver organoids also exhibited CYP3A4 activity, measured using the P450-Glo™ CYP3A4 Assay and Screening System. This activity was (B) inhibited and (C) induced in a dose-dependent manner following 72 hours of exposure to ketoconazole and calcitriol, respectively (mean ± SD, n = 2). Statistical significance was determined using ordinary one-way ANOVA ****p < 0.0001, ***p = 0.001, **p = 0.0074, *p = 0.0149.

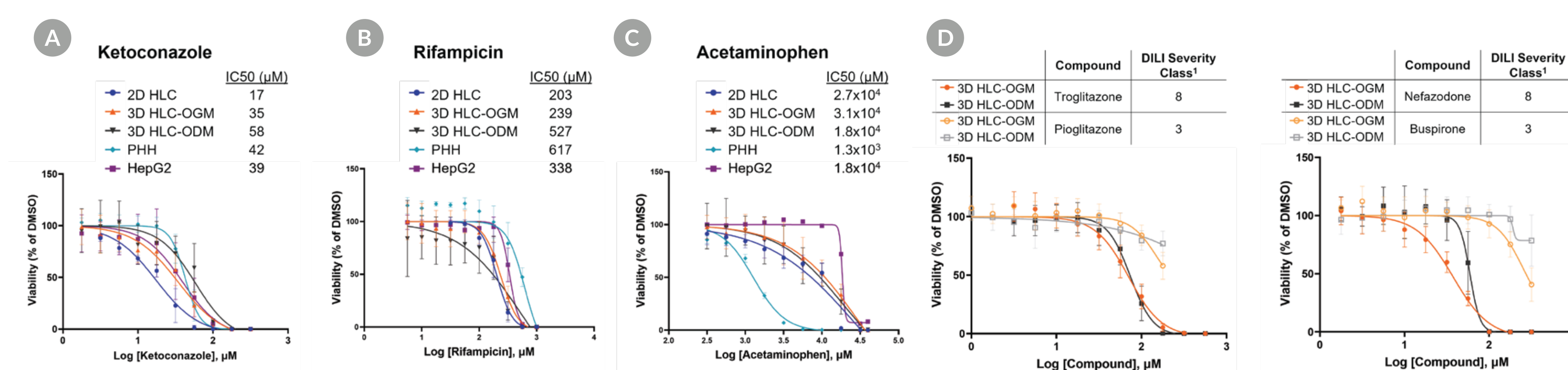


FIGURE 6. HLCs and HLC-Derived Organoids Are Sensitive to Compound-Induced Hepatotoxicity and Can Distinguish Between Related Compounds with Different DILI Severities

HLCs and HLC-derived organoids showed high, moderate, or low dose-dependent sensitivity to (A) ketoconazole, (B) rifampicin, and (C) acetaminophen, respectively. HLC-derived liver organoids also exhibited dose-dependent sensitivity to (D) troglitazone and nefazodone and were less sensitive to their related compounds pioglitazone and buspirone, respectively. These results align with previously reported drug-induced liver injury (DILI) severity class classifications¹ for these compounds, demonstrating the suitability of these hepatic models in ranking the relative toxicity of compounds.

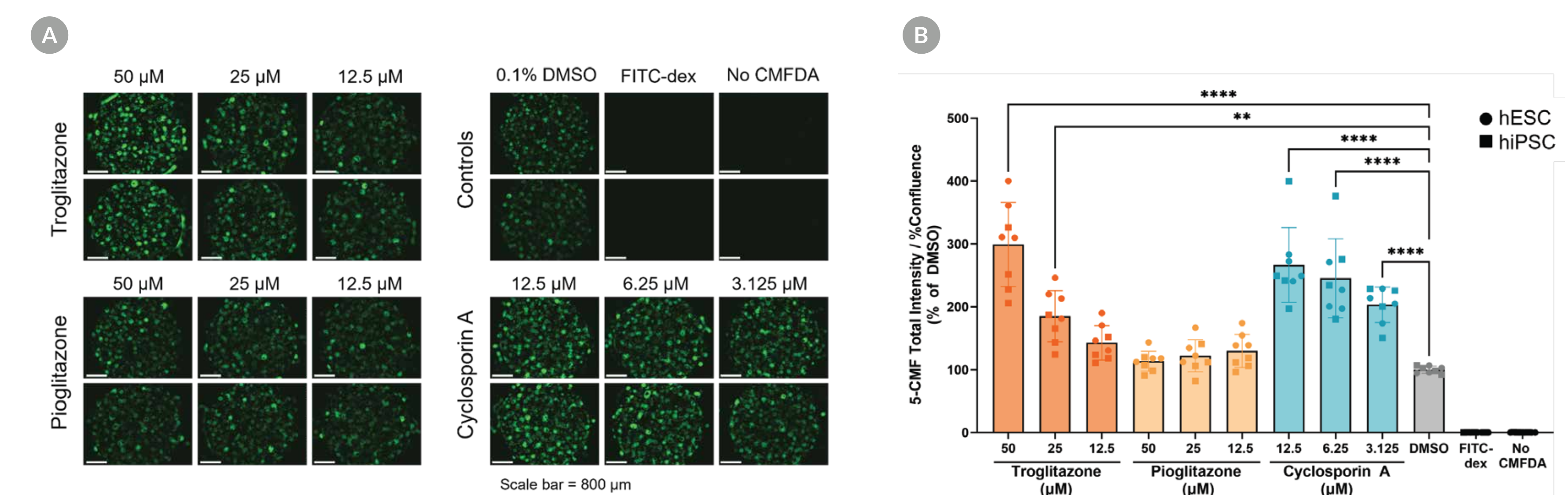


FIGURE 6. HLC-Derived Organoids Respond to Bile Efflux Inhibitors and Distinguish Between Cholestatic and Non-Cholestatic Compounds

Differentiated HLC-derived organoids were incubated with either a FITC-dextran control (FITC-dex), or with 5-chloromethylfluorescein diacetate (CMFDA) in the presence of a DMSO vehicle control, compounds that inhibit bile efflux, or a non-cholestatic control. (A) Organoids were able to cleave cell permeant, non-fluorescent CMFDA to produce membrane-impermeable fluorescent 5-chloromethylfluorescein (5-CMF), as shown by green fluorescence in cells. (B) In the presence of bile efflux inhibitor cyclosporin A and cholestatic compound troglitazone, 5-CMF efflux into supernatant or organoid lumens was decreased, such that green fluorescence intensity retained in the cells was significantly higher than the vehicle control. This was not the case for pioglitazone, a non-cholestatic compound, where intracellular green intensity was comparable to the vehicle control. Data represents mean ± SD, n = 4 technical replicates per dose across 2 independent experiments using 2 donors per experiment. Statistical significance was determined using one-way ANOVA, ****p < 0.0001, **p = 0.001.

SUMMARY

- STEMdiff™ Hepatocyte Kit supports the efficient generation of hPSC-derived hepatocyte-like cells that exhibit mature hepatic phenotypes and functionality.
- Hepatic progenitor- and hepatocyte-like cell-derived liver organoids, established using STEMdiff™ Hepatic Organoid Growth Medium, are compatible with long-term expansion, cryopreservation, and further differentiation to increase hepatic maturity.
- Hepatocyte-like cells and differentiated hPSC-derived liver organoids are sensitive to hepatotoxic compounds.
- Differentiated hPSC-derived liver organoids can distinguish between related compounds with differing DILI severities, and can be used to model effects on bile acid efflux transporters.

References

¹Chen et al. Drug Discov Today (2016) 21(4):648-653.