Supporting Information

Label-free Evaluation of Maturation and Hepatotoxicity of Human iPSC-derived Hepatocytes using Hyperspectral Raman Imaging

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Table S1 The primary antibodies used in this study

Antigen	Type	Company	Catalog number	Dilution
NANOG	Mouse	Santa Cruz Biotechnology	sc-293121	1:300
CXCR4	Mouse	Santa Cruz Biotechnology	sc-53534	1:300
HNF4α	Rabbit	Cell Signaling Technology	CST3113	1:300
CYP3A4	Mouse	Sigma-Aldrich	SAB5300118	1:1000
ALB	Goat	Bethyl laboratories	A80-129A	1:500
CK-19	Rabbit	Abcam	ab52625	1:500
Cyt *c*	Mouse	Santa Cruz Biotechnology	Sc-13561	1:500

Table S2 The secondary antibodies used in this study

Antigen	Type	Fluorophore	Company	Catalog number	Dilution
Mouse IgG	Goat	Alexa Fluor 488	Invitrogen	A11001	1:500
Mouse IgG	Goat	Alexa Fluor 594	Invitrogen	A11005	1:500
Rabbit IgG	Goat	Alexa Fluor 488	Invitrogen	A11008	1:500
Rabbit IgG	Goat	Alexa Fluor 594	Invitrogen	A11012	1:500
Goat IgG	Donkey	Alexa Fluor 594	Invitrogen	A11058	1:500

Table S3 The primers used for real-time RT-PCR in this study

Gene Symbol	Primers (forward/reverse; 5' to 3')
SOX2	GGCAGCTACAGCATGATGATGCAGGAGC/CTGGTCATGGAGTTGTACTGCAGG
SOX17	GTGGACCGCACGGAATTTG/GAGGCCCATCTCAGGCTTG
HNF4α	CGTCATCGTTGCCAACACAAT/GGGCCACTCACACATCTGTC
CYP3A4	AAGTCGCCTCGAAGATACACA/AAGGAGAGAACACTGCTCGTG
ALB	GCACAGAATCCTTGGTGAACAG/GCACAGAATCCTTGGTGAACAG
GAPDH	GGTGGTCTCCTCTGACTTCAACA/GTGGTCGTTGAGGGCAATG

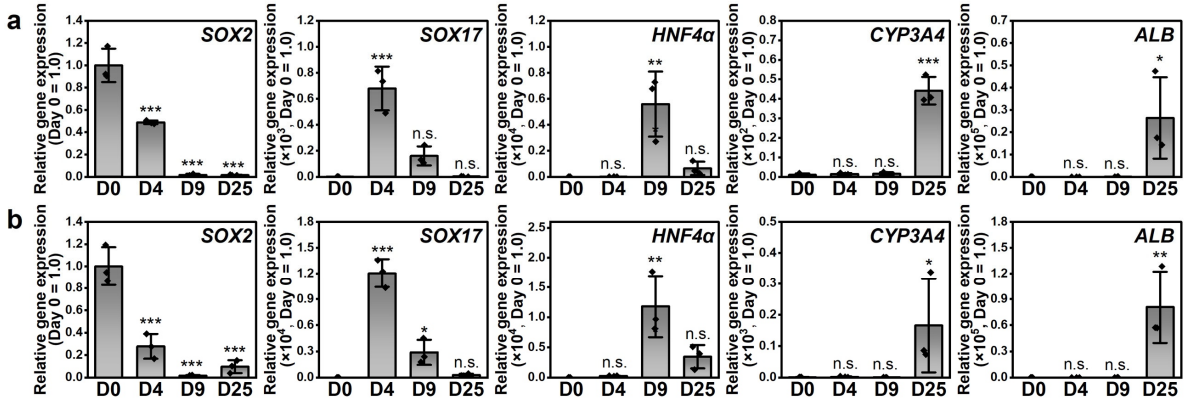

Figure S1 The gene expression levels of *SOX2*, *SOX17*, *HNF4α*, *CYP3A4*, and *ALB*. (a) hiPSCs and their derivatives cultured in plastic plates. (b) hiPSCs and their derivatives cultured in quartz dishes.

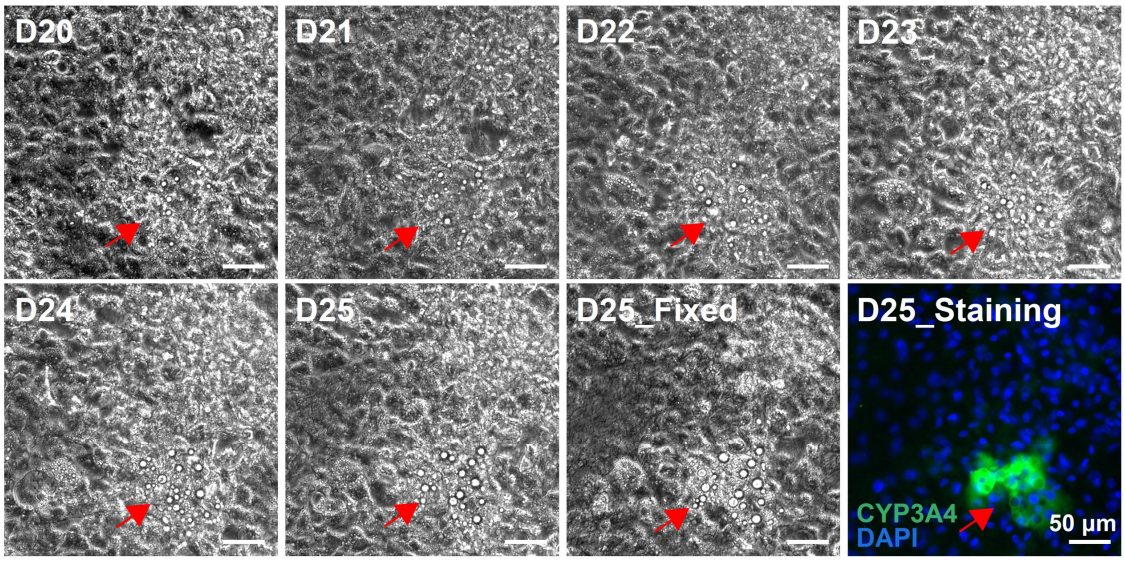

Figure S2 Time-course change of lipid-rich colony from D20 to D25 following immunofluorescence staining of CYP3A4. This result indicates the potential of using lipid as an early marker of CYP-positive HLCs. Red arrows indicate the same position at different time points. Scale bars, 50 μm.

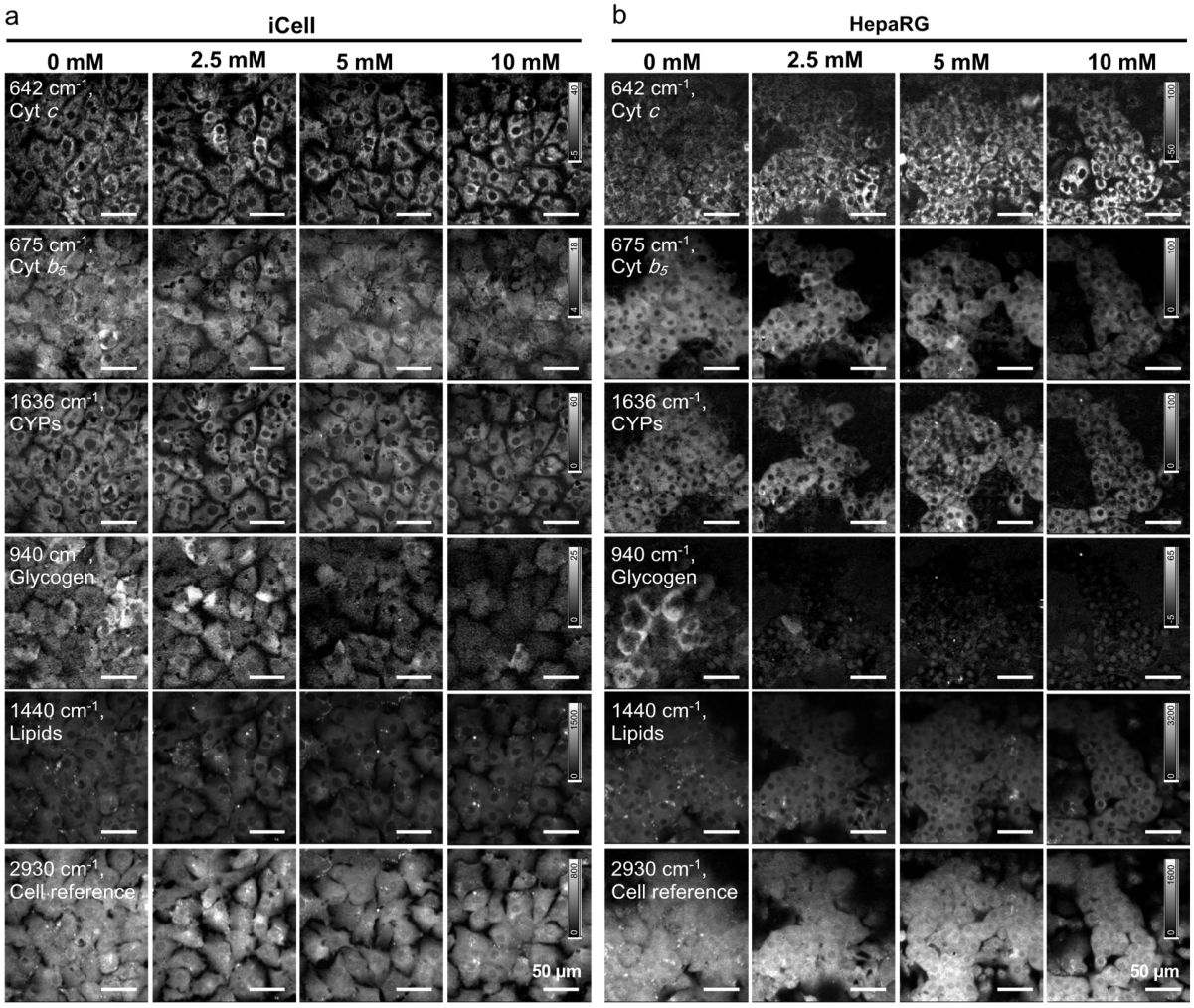

Figure S3 Screening hepatotoxicity in iCell hepatocytes and HepaRG cells using Raman imaging. 0-10 mM APAP was applied to iCell hepatocytes (a) and HepaRG cells (b) culture. Reconstructed Raman images indicating cellular components at 642, 675, 1636, 940, 1440, and 2930 cm^{-1} are shown. Scale bars, 50 µm.