

Supplementary Material

Metabolically Improved Stem Cell Derived Hepatocyte-Like Cells Support HBV Life Cycle and Are a Promising Tool for HBV Studies and Antiviral Drug Screenings

Tine Tricot ^{1,*†}, Hendrik Jan Thibaut ^{2,3,*†}, Kayvan Abbasi ², Ruben Boon ^{1,4}, Nicky Helsen ^{1,5}, Manoj Kumar ¹, Johan Neyts ^{2†} and Catherine Verfaillie ^{1,*†}

¹ Stem Cell Institute, Rega Institute KU Leuven, 3000 Leuven, Belgium; ruben.boon@kuleuven.be (R.B.); nicky.helsen@ismar.com (N.H.); manoj.kumar@kuleuven.be (M.K.)

² Department of Microbiology, Immunology and Transplantation, Virology and Chemotherapy, Rega Institute KU Leuven, 3000 Leuven, Belgium; kayvan.abbasi@kuleuven.be (K.A.); johan.neyts@kuleuven.be (J.N.)

³ Department of Microbiology, Immunology and Transplantation, Translational Platform Virology and Chemotherapy (TPVC), Rega Institute KU Leuven, 3000 Leuven, Belgium

⁴ Laboratory for Functional Epigenetics, Department of Human Genetics, Rega Institute KU Leuven, 3000 Leuven, Belgium

⁵ Ismar Healthcare NV, 2500 Lier, Belgium

* Correspondence: tine.tricot@kuleuven.be (T.T.); Hendrikjan.thibaut@kuleuven.be (H.J.T.); catherine.verfaillie@kuleuven.be (C.V.); Tel.: +32-16-37-71-09 (T.T.); +32-16-32-16-82 (H.J.T.); +32-16-37-26-54 (C.V.)

† These authors contributed equally to this work.

Supplementary

Table.....1

Supplementary

Figures.....2

Supplementary Tables

Table S1. Primer list.

GENE	FORWARD PRIMER	REVERSE PRIMER
<i>RPL19</i>	ATTGGTCTCATTGGGGTCTAAC	AGTATGCTCAGGCTTCAGAAGA
<i>ALB</i>	ATGCTGAGGCAAAGGATGTC	AGCAGCAGCACGACAGAGTA
<i>AAT</i>	AGGGCCTGAAGCTAGTGGAT	TCCTCGGTGTCCTTGACTTC
<i>HNF4</i>	ACTACGGTGCCTCGAGCTGT	GGCACTGGTTCCTCTTGTCT
<i>NTCP</i>	ATCGTCCTCAAATCCAAACG	CCACATTGATGGCAGAGAGA

Table S2. Antibody list.

ANTIBODY	CATALOG NUMBER	COMPANY	DILUTION
HNF4	Ab41898	Abcam	1:200
NTCP	HPA042727	Sigma-Aldrich	1:500
HBcAg	B0586	Dako	1:750
HBsAg	SAB 4700767-100UG	Sigma-Aldrich	1:500
ALEXA 555 DONKEY ANTI-RABBIT	Thermo Fisher	A31572	1:500
ALEXA 488 DONKEY ANTI-RABBIT	Thermo Fisher	A21206	1:500
ALEXA 555 DONKEY ANTI-MOUSE	Thermo Fisher	A31570	1:500
ALEXA 488 DONKEY ANTI-MOUSE	Thermo Fisher	A21202	1:500
ISOTYPE RABBIT IgG	BD Pharmingen	550875	Used as control in same concentration as primary antibody
ISOTYPE MOUSE IgG2a	Sigma-Aldrich	M9144-1MG	Used as control in same concentration as primary antibody

Supplementary Figures

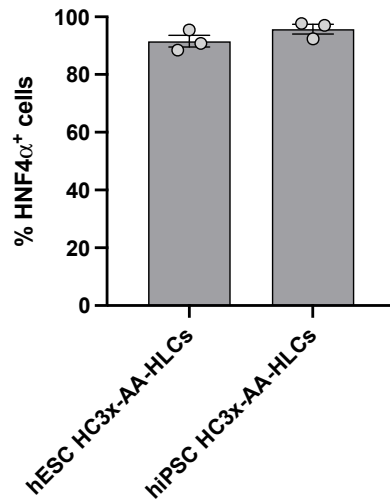


Figure S1. Purity of the hESC and hiPSC HC3x-AA-HLC differentiation. Quantification of immunofluorescence staining for HNF4 α ⁺ hESC and hiPSC HC3x-AA-HLCs. All data is shown as mean \pm SEM.

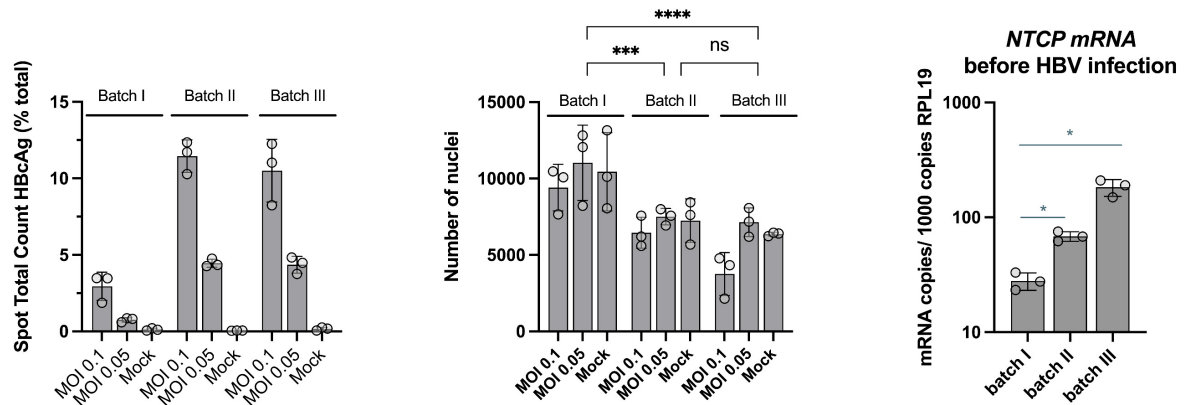


Figure S2. Variability in HBV infection efficiency is possibly caused by differences in cell density and NTCP expression levels. Quantification of immunofluorescence staining for HBcAg and cell number in mock and HBV-infected HC3x-AA-HLCs (MOI 0.1 and 0.05), as well as gene expression analysis for NTCP in 3 different experiments. RPL19 was used for normalization. All data is shown as mean \pm SEM.