Supplementary material

Supplementary Figure 1

Retrorsine improves liver repopulation across different liver chimeric models

a) Retrorsine has no effect on overall survival during NTBC cycling and hepatocyte repopulation. Log-rank (Mantel-Cox) test between groups of 13 mice.

b) Groups of 5 non-preconditioned FNRG mice received various doses of AAV8-hOSM or AAV8-FLuc negative control before transplantation of human fetal hepatoblasts. Low doses of AAV8-hOSM result in higher serum hAlb levels. Median ± range hAlb levels, t-test of time points, * p<0.05 of both 3x10^8 and 1x10^9 hOSM groups compared to FLuc, ** p<0.01 of 3x10^8 hOSM compared to FLuc.

c) In the presence of AAV8-hOSM, groups of 5 retrorsine-preconditioned FNRG mice transplanted with fetal hepatoblasts reach higher serum hAlb levels than vehicle control animals. Median ± range hAlb levels, p=0.0014 between groups by mixed design ANOVA.

d) Retrorsine preconditioning of PHH2 transplanted TK-NOG mice results in higher peak serum hAlb values. Symbols are individual mice, median ± range, t-test.

e) Hemizygous alb-uPA mice treated with retrorsine (squares) prior to PHH2 transplantation trend towards higher peak serum hAlb levels and then maintain hAlb above 10^3 µg/ml for longer duration than mice receiving vehicle (circles). Symbols are individual mice, p=0.0003 between groups by 2-way ANOVA.
Supplementary Figure 2

Retrorsine increases mouse hepatocyte Ki67 expression after restarting NTBC

a) Experimental schematic in which FNRG mice received either retrorsine or vehicle prior to NTBC withdrawal and reintroduction. Livers from untransplanted FNRG mice were harvested at indicated times.

b) Staining for the cell cycle marker Ki67 showed few cycling hepatocytes in either group one day after restarting NTBC but became more abundant in retrorsine than vehicle preconditioned livers two days later.

c) Quantification of Ki67+ hepatocytes per high power field (HPF) showed retrorsine to increase the number of Ki67+ hepatocytes after restarting NTBC. Median ± range of 10 HPF of two livers per group.

d) Heatmap of mouse growth factor protein levels in day 14 livers showed several factors being affected by retrorsine preconditioning. Fold change over vehicle control mice, n=3 mice per group, * p<0.05.
Supplementary Figure 3

Gene expression profile of mpPHH is similar to cryopreserved PHH

RNAseq analysis of 2 cryopreserved PHH (PHH3) and 3 freshly isolated mpPHH3 from 5 humanized mice. Comparison of transcripts of Phase I detoxification genes (i.e. CYP450), Phase II detoxification genes and various liver-specific genes (nuclear receptors, liver-enriched transcription factors).
Supplementary Figure 4

Long-term mpPHH cultures are stable for at least 8 weeks

a) Seeding density titration of mpPHH for long-term culture (8 weeks) as shown by secreted hAlb levels over time. Media were changed on days 2, 4 and 7 for a total of 8 weeks. hAlb levels were measured in culture supernatants every seventh day. Numbers in the legend indicate number of cells in thousands seeded in 12-well plates. Data from biological duplicates are represented as mean ± s.d.

b) Representative low and high magnification images from wells seeded with 1 million cells after 7 days and 56 days in culture.

c) Hepatocyte markers measured in culture supernatants after seeding mpPHH on collagen-coated 12-well plates. Media were changed every two days. Human albumin (hAlb), human transferrin (hTF), human alpha-antitrypsin (hAAT) and urea were measured for up to 29 days after seeding. Data from four biological replicates are represented as mean ± s.d.
Supplementary Figure 5

Retrorsine can rescue non-plateable PHH donors

a) Plateable PHH2 and non-plateable PHH8 were transplanted into groups of 2-3 FNRG mice, with retrorsine improving hAlb levels 22-fold in mice transplanted with PHH8. Median ± range serum hAlb levels.

b) Brightfield images 3 days after plating illustrate that non-plateable PHH8 only formed cultures ex vivo whereas plateable donor PHH2 also formed in vitro cultures.
Supplementary Figure 6

mpPHH are suitable for high-throughput applications in 384-well plates

a) mpPHH were seeded in a 384-well plate. Brightfield images of confluent cultures.

b) Cells seeded in 384-well plate were infected with HBV in the presence or absence of HBV entry inhibitor Myrcludex B (500 nM). After washing (1 dpi and 3 dpi) the cells were cultured for an additional 7 days. At 10 dpi culture supernatants were harvested and cells were fixed and stained for HBcAg (red) and nuclei (blue). HBeAg levels in culture supernatants were measured using a chemiluminescence (CLIA) assay. Data from four biological replicates are represented as mean ± s.d.
Figure S6

a) 384-well

b) Day 0 1 3 10

endpoint measurements

HBeAg (PEI U/ml)

uninfected

+ HBV

+ HBV + MyrB

HBV - + +

MyrB - - +
Supplementary Figure 7

Cultured mpPHH can be transduced, mobilized and then used to form 3D spheroid cultures

a) mpPHH in 2D cultures were detached and then transduced with RFP-lentiviruses in suspension to form 3D spheroid cultures. Brightfield and RFP fluorescence imaging demonstrates low transduction efficiency.

b) mpPHH in 2D cultures were transduced with RFP-lentiviruses then detached and used to form 3D spheroid cultures. Brightfield and RFP fluorescence imaging indicates high transduction efficiency.

c) Z-stacks of spheroids made by transduced mpPHH (from panel b).

d) Cell seeding titration of RFP-transduced mpPHH (in thousands cells) used to form single spheroid cultures in 96-well plates.
Figure S7

(a) lentiviral transduction

(b) transduced hepatocytes

(c) merged images

(d) varied resolutions
Supplementary Figure 8

Human NuMA staining of a liver slice from a humanized mouse engrafted with RFP-transduced mpPHH

a) Livers from mice from Figure 5f were processed for immunohistochemistry. Areas of human (h) and murine (m) cells are separated with lines. An area with human hepatocytes is shown at high magnification.

b) Cells from Figure 5f were cultured for 2 weeks with media changes every 2 days. A representative image of RFP expression is shown on day 15 post-seeding.
Figure S8

(a) [Image of a histological section]

(b) re-plated mpPHH (RFP+)[Image of re-plated cells]