Mouse liver repopulation with hepatocytes generated from human fibroblasts

Saiyong Zhu¹, Milad Rezvani², Jack Harbell³, Aras N. Mattis²,⁴,⁵, Alan R. Wolfe⁶, Leslie Z. Benet⁶, Holger Willenbring²,³,⁴ & Sheng Ding¹,²,⁷

Human induced pluripotent stem cells (iPSCs) have the capability of revolutionizing research and therapy of liver diseases by providing a source of hepatocytes for autologous cell therapy and disease modelling. However, despite progress in advancing the differentiation of iPSCs into hepatocytes (iPSC-Heps) in vitro¹–³, cells that replicate the ability of human primary adult hepatocytes (aHeps) to proliferate extensively in vivo have not been reported. This deficiency has hampered efforts to recreate human liver diseases in mice, and has cast doubt on the potential of iPSC-Heps for liver cell therapy. The reason is that extensive post-transplant expansion is needed to establish and sustain a therapeutically effective liver cell mass in patients, a lesson learned from clinical trials of aHep transplantation⁴. Here, as a solution to this problem, we report the generation of human fibroblast-derived hepatocytes that can repopulate mouse livers. Unlike current protocols for deriving hepatocytes from human fibroblasts, ours did not generate iPSCs but cut short reprogramming to pluripotency to generate an induced multipotent progenitor cell (iMPC) state from which endoderm progenitor cells and subsequently hepatocytes (iMPC-Heps) could be efficiently differentiated. For this purpose we identified small molecules that aided endoderm and hepatocyte differentiation without compromising proliferation. After transplantation into an immune-deficient mouse model of human liver failure, iMPC-Heps proliferated extensively, and acquired levels of hepatocyte function similar to those of aHeps⁵. Unfractionated iMPC-Heps did not form tumours, most probably because they never entered a pluripotent state. Our results establish the feasibility of significant liver repopulation of mice with human hepatocytes generated in vitro, which removes a longstanding roadblock on the path to autologous liver cell therapy.

Current protocols for directed hepatocyte differentiation of iPSCs (or human embryonic stem cells; ESCs) produce cells that express many functions of human primary adult hepatocytes (aHeps)¹–³. Some functions of iPSC/ESC-Heps, such as cytochrome P450 (CYP450) expression, are underdeveloped, whereas others, such as albumin (ALB) synthesis, are near normal. Human serum ALB (HSA) levels from transplantation of these cells into mice have been reported. All protocols for hepatocyte differentiation of iPSCs/ESCs, numerous results from transplantation of these cells into mice have been reported. All of these studies, even those in which transplanted iPSC/ESC-Heps had a growth advantage, failed to reach HSA levels above 2 μg ml⁻¹ (refs 5–7), which is more than 1,000-fold lower than what has been achieved with aHeps⁵ and in our experience reflects less than 0.05% liver repopulation (data not shown). These disappointing results have been largely attributed to failure of iPSC/ESC-Heps to proliferate after transplantation.

Reprogramming of somatic cells to a stable pluripotent state followed by differentiation into another cell type is a complicated process. Alternatively, the developmentally plastic state established soon after overexpression of the reprogramming factors can be harnessed for lineage conversion. Using this approach we and others previously induced human fibroblasts to assume a multipotent progenitor cell (iMPC) state that allowed efficient differentiation into myeloid⁶ or smooth muscle and endothelial cells⁶,⁷. Because these iMPC derivatives could proliferate extensively, we reasoned that this method could be used to generate hepatocytes that were not compromised by growth arrest.

To investigate this possibility we transduced 10⁴ human fibroblasts with retroviruses expressing OCT4, SOX2 and KLF4 (ref. 12) and reprogrammed them for reprogramming into endoderm in medium containing established growth factors and the small molecule CHIR99021 (CHIR)¹–³ (Fig. 1). Only 14 days later, we detected expression of the endoderm-specific genes SOX17 and FOXA2 by quantitative PCR with reverse transcription (qRT–PCR) (Extended Data Fig. 1a). While only about 20 SOX17-positive and FOXA2-positive colonies formed under these conditions.

Figure 1 Protocol for stepwise iMPC-Hep generation. Reprogramming of human fibroblasts to endoderm was initiated in medium containing CHIR (glycogen synthase kinase 3β inhibitor), dilauroyl phosphatidycholine (DLPC; liver receptor homologue-1 agonist), the epigenetic modifiers sodium butyrate (NaB; histone deacetylase inhibitor), Parnate (Par; lysine-specific demethylase 1 inhibitor) and RG108 (RG; DNA methyltransferase inhibitor), and epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). To promote reprogramming, EGF and bFGF were replaced with activin A. Individual iMPC-EPC colonies were expanded in medium containing CHIR, EGF, bFGF and A83 (transforming growth factor-β type I receptor inhibitor). For hepatocyte specification, medium containing bFGF, A83, bone morphogenetic protein 4 (BMP4), dexamethasone (Dex), hepatocyte growth factor (HGF), oncostatin M (OSM) and the Notch inhibitor Compound E (C-E) was used.
conditions, exposing the cells to additional small molecules known to promote reprogramming\cite{13,14} increased the number of colonies to more than 80 (Extended Data Fig. 1b–d and Supplementary Table 1).

Next we investigated whether de novo endoderm differentiation was preceded by a pluripotent state. We found no expression of the pluripotency-specific genes OCT4 and NANOG even at the earliest stages of the reprogramming process (Extended Data Fig. 1e). Because avoiding a pluripotent state decreases the cells’ tumour risk, we confirmed this result by TRA-1-60 (ref. 15) flow cytometry at the end of the reprogramming process (Extended Data Fig. 1f, g). In addition we monitored cultures undergoing reprogramming for FOXA2-positive cells, referred to as iMPC-derived endoderm progenitor cells (iMPC-EPCs), and NANOG-positive cells by immunostaining and flow cytometry (Extended Data Fig. 2a). We found FOXA2-positive cells as early as 16 days after initiating reprogramming, whereas NANOG-positive cells were always absent (Extended Data Fig. 2b–d). We also used doxycycline-inducible lentiviruses expressing OCT4, SOX2 and KLF4 to compare the dynamics of reprogramming to endoderm versus pluripotency (Extended Data Fig. 3a). We detected iMPC-EPC colonies in transduced cultures grown under iMPC-EPC reprogramming conditions for 21 days after only 7 days of treatment with doxycycline. In contrast, generation of iPSCs required treating the cultures with doxycycline for 14 days and growing them under iPSC reprogramming conditions for 30 days (Extended Data Fig. 3b). Our findings that human fibroblasts reprogram into iMPC-EPCs faster than into iPSCs and without expressing pluripotency markers show that our protocol does not produce a pluripotent intermediate stage, which confirms previous results from shortcutting reprogramming to pluripotency for lineage conversion\cite{16,17}.

We also determined whether iMPC-EPCs could be expanded in vitro, a prerequisite for producing the large quantities needed for human liver cell therapy. We found that combining CHIR with A83-01 (A83)\cite{18} increased iMPC-EPC colony size (Fig. 1 and Extended Data Fig. 4a). The addition of epidermal growth factor and basic fibroblast growth factor caused further expansion and facilitated passaging for more than 25 times, producing more than $10^{16}$ iMPC-EPCs from a single colony (Fig. 2a, b, Extended Data Fig. 4b and Supplementary Table 1). These cells showed high viability after cryopreservation (data not shown).

Expanding iMPC-EPCs maintained endoderm differentiation, as demonstrated by positive immunostaining for FOXA2 and SOX17 and negative immunostaining for NANOG (Fig. 2a and Extended Data Fig. 4c). Expanding iMPC-EPCs acquired hepatocyte nuclear factor 4α (HNF4α) expression (Fig. 2a and Extended Data Fig. 4d), suggesting further specification. To define their stage of differentiation, we compared iMPC-EPCs with ESC-derived definitive endoderm cells (DECs) and primitive gut-tube endoderm cells (GECs)\cite{19} (Fig. 2c). We found that iMPC-EPCs resembled ESC-GECs, except for a lack of OCT4 and NANOG gene expression. iMPC-EPCs also lacked expression of the ectoderm-specific and mesoderm-specific genes PAX6 and BRY, suggesting commitment to endoderm differentiation. Further analyses showed that iMPC-EPCs had a propensity for differentiating into liver and pancreas but not into lung or intestine (Extended Data Fig. 4e, f and data not shown). These results establish the feasibility of using human fibroblasts to generate endoderm cells that share many characteristics with previously reported ESC/iPSC-derived endodermal progenitor cell lines\cite{20,21}, but seem more lineage restricted and never entered a pluripotent state.

To further differentiate iMPC-EPCs into iMPC-Heps, we cultured them in medium containing factors that have been reported to drive the hepatic differentiation of iPSC-DECs\cite{22,23} (Fig. 1). These factors were effective in inducing expression of the fetal hepatocyte marker $\alpha$-fetoprotein, but few cells expressed the more mature markers ALB and $\tau_2$-antitrypsin (AAT) (Extended Data Fig. 5a). To improve hepatocyte differentiation, we screened small molecules for inducers of ALB gene expression, of which A83 and the Notch inhibitor Compound E\cite{24} were effective (Extended Data Fig. 5b). Because transforming growth factor-β and Notch signalling direct bipotential embryonic liver progenitor cells towards a biliary fate\cite{25,26}, our results suggest that inhibiting biliary differentiation promotes hepatocyte differentiation.
Like aHeps, iMPC-Heps were polygonal, were occasionally binucleate, and expressed the hepatocyte markers HNF4α, ALB, AAT and cytokeratin 18 (Fig. 3a). iMPC-Heps also showed hepatocyte functions such as glycogen storage, lipid uptake and storage, and urea production (Extended Data Fig. 6a, b). Gene expression analysis showed that iMPC-Heps generally resembled human primary fetal hepatocytes (fHeps) (Fig. 3b), although some cells were less differentiated (Fig. 3c and Supplementary Table 1). Analysis of ALB secretion and CYP450 activities confirmed that iMPC-Heps were less mature than aHeps, but also showed that iMPC-Heps were more differentiated than iPSC-Heps generated as reported previously3-5 (Fig. 3d, e and Extended Data Fig. 6c). The medium used for iMPC-EPC/Hep generation did not produce iPSC-Heps with improved function, which underscores the importance of reprogramming-induced developmental plasticity in this process (Extended Data Fig. 6d).

To test whether iMPC-Heps can expand after transplantation, we transplanted 10⁶ cells into FRG mice, an immune-deficient mouse model of human tyrosinaemia type I (ref. 8). The liver injury caused by this disease creates a growth advantage for differentiated hepatocytes but not for immature liver progenitor cells. Liver repopulation of FRG mice therefore requires both mature hepatocyte function and the ability to proliferate. To detect expansion of the transplanted iMPC-Heps, we measured HSA levels monthly for more than nine months. The earliest we could detect HSA was two months after transplantation (Fig. 4a), when levels were at most 140 ng ml⁻¹, but they increased continuously, reaching levels of up to 104 μg ml⁻¹ six months later. By this time, HSA levels were tenfold higher in control FRG mice transplanted with 10⁶ aHeps. The delayed onset but parallel upward trend of HSA levels in iMPC-Hep-transplanted mice, in comparison with control mice, suggested that iMPC-Heps were inferior to aHeps in engraftment efficiency and the need for post-transplant maturation, but not in the ability to proliferate. Indeed, we found that although iMPC-Heps generated significantly fewer repopulating nodules than aHeps (data not shown), these nodules grew markedly between three and nine months after transplantation (Extended Data Fig. 7a). Moreover, iMPC-Heps still proliferated nine months after transplantation (Fig. 4b). So far we have observed a maximum nodule size of 4,000 iMPC-Heps and a liver repopulation level of 2% (Extended Data Fig. 7b, c).

To determine whether iMPC-Heps matured after transplantation, we compared the global gene expression profiles of transplanted iMPC-Heps and aHeps. For this purpose we isolated nodules of iMPC-Heps and aHeps by laser-capture microscopy (Extended Data Fig. 7d, e) and analysed their RNA with microarrays. We found that iMPC-Heps and aHeps clustered closely together—very few genes were differentially expressed, none of which were of known importance for hepatocyte function (Fig. 4c, Extended Data Fig. 8a–e and Supplementary Table 2). We also compared cultured iMPC-Heps and freshly isolated aHeps, and found marked differences in gene expression between these two cell types, which illustrates the extensive maturation that iMPC-Heps underwent after transplantation. In fact, before transplantation, the gene expression profile of iMPC-Heps resembled that of iPSC-Heps. We confirmed the microarray results by qRT–PCR and immunostaining (Fig. 4d and Extended Data Fig. 9a–c). In addition we determined whether maturation of gene expression translated into normal function by measuring debrisoquine hydroxylation—a unique function of human hepatocytes executed by CYP2D6 (ref. 21)—in mice repopulated to similar levels with iMPC-Heps or aHeps. We found no difference in plasma 4-hydroxy-debrisoquine levels between these mice, which shows that CYP2D6 underwent maturation in iMPC-Heps from negligible expression levels in vitro to normal activity in vivo (Figs 3b and 4e). In accord with a need for post-transplant maturation of iMPC-Heps, which is reminiscent of iPSC/ESC-derived pancreatic β-cells22, we found that iMPC-Hep transplantation improved the survival of mice with chronic liver failure, but not that of those with acute liver failure (Extended Data Fig. 10a, b).

Finally, we ruled out fusion with mouse hepatocytes as the reason for post-transplant maturation and proliferation of iMPC-Heps (Fig. 4f), and investigated the origin of dysplastic nodules observed in some iPSC-Hep and aHep recipients (Extended Data Fig. 10c). Absence of differentiation-independent, human-specific β₂-microglobulin expression showed that these nodules originated from mouse cells, thus representing a known complication of tyrosinaemia type I (Extended Data Fig. 10d)23.

Our results show that iMPC-Heps are not affected by limitations of iPSC/ESC-Heps generated with current protocols, particularly deficiencies in in vivo efficacy and safety. Although many aspects of iMPC-Hep generation and transplantation remain to be explored and improved, the fact that these cells can fully mature and proliferate for months after
transplantation establishes them as promising candidates for in vivo modelling and autologous therapy of human liver diseases.

METHODS SUMMARY

Generation of iMPC-Heps from human fibroblasts. After retroviral transduction, human fibroblasts were grown in reprogramming initiation medium for 7 days, followed by culture in endoderm differentiation medium for 14–21 days. iMPC-EPC colonies were selected at day 21–28 and grown on mouse embryonic fibroblasts in endoderm expansion medium for 1–4 months. For differentiation into iMPC-Heps, iMPC-EPCs were first expanded for 4–5 days, followed by culture in hepatocyte differentiation medium for 4 days and then in hepatocyte maturation medium for 7–10 days. Media were supplemented with the growth factors and small molecules indicated in Fig. 1.

iMPC-Hep transplantation and analysis. iMPC-Heps were injected intrasplenically into FRG mice preconditioned by withdrawal of the drug 2-(2-nitro-4-fluoromethylbenzyl)-1,3-cyclohexanedione (NTBC) and retro-orbital injection of an adenovirus expressing urokinase plasminogen activator. Mice underwent iMPC-Hep transplantation and analysis.

Online Content

Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 12 June 2013; accepted 10 January 2014.

Published online 23 February 2014.

1. Si-Tayeb, K. et al. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. Hepatology 51, 297–305 (2010).
2. Rashid, S. T. et al. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. J. Clin. Invest. 120, 3127–3136 (2010).
3. Ma, X. et al. Highly efficient differentiation of functional hepatocytes from human induced pluripotent stem cells. Stem Cells Transl. Med. 2, 409–419 (2013).
4. Puppi, J. et al. Improving the techniques for human hepatocyte transplantation: report from a consensus meeting in London. Cell Transplant. 21, 1–10 (2012).
5. Liu, H., Kim, Y., Sharkis, S., Marchionni, L. & Jang, Y. Y. In vivo liver regeneration potential of human induced pluripotent stem cells from diverse origins. Sci. Transl. Med. 3, 52ra39 (2011).
6. Basma, H. et al. Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. Gastroenterology 136, 990–999 (2009).
7. Woo, D. H. et al. Direct and indirect contribution of human embryonic stem cell-derived hepatocyte-like cells to liver repair in mice. Gastroenterology 142, 602–611 (2012).
8. Azuma, H. et al. Robust expansion of human hepatocytes in Fah<sup>-/-</sup>/Rag2<sup>-/-</sup>/ Il2rg<sup>-/-</sup> mice. Nature Biotechnol. 25, 903–910 (2007).
9. Szabo, E. et al. Direct conversion of human fibroblasts to multilineage blood progenitors. Nature 468, 521–526 (2010).
10. Kurnit, L. et al. Conversion of human fibroblasts to angioblast-like progenitor cells. Nature Methods 10, 77–83 (2013).
11. Li, J. et al. Conversion of human fibroblasts to functional endothelial cells by defined factors. Arterioscler. Thromb. Vasc. Biol. 33, 1366–1375 (2013).
12. Takahashi, K. et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872 (2007).
13. Zhu, S. et al. Reprogramming of human primary somatic cells by OCT4 and chemical compounds. Cell Stem Cell 7, 651–655 (2010).
14. Lee, J. M. et al. Nuclear-receptor-dependent phosphatidylcholine pathway with anti-diabetic effects. Nature 474, 506–510 (2011).
15. Chan, E. M. et al. Live cell imaging distinguishes bona fide human iPSCs from partially reprogrammed cells. Nature Biotechnol. 27, 1033–1037 (2009).
16. Li, W. et al. Rapid induction and long-term self-renewal of primitive neural precursors from human embryonic stem cells by small molecule inhibitors. Proc. Natl Acad. Sci. USA 108, 8299–8304 (2011).
17. Wang, P., Rodriguez, R. T., Wang, J., Ghodasara, A. & Kim, S. K. Targeting SOX17 in human embryonic stem cells creates unique strategies for isolating and analyzing developing endoderm. Cell Stem Cell 8, 335–346 (2011).
18. Cheng, X. et al. Self-renewing endodermal progenitor lines generated from human pluripotent stem cells. Cell Stem Cell 10, 371–384 (2012).
19. Clotman, F. et al. Control of liver cell fate decision by a gradient of TGF β signaling modulated by Oncuit transcription factors. Genes Dev. 19, 1849–1854 (2005).
20. Kodama, Y., Hijkata, M., Kagayama, R., Shimotohno, K. & Chiba, T. The role of notch signaling in the development of intrahepatic bile ducts. Gastroenterology 127, 1775–1786 (2004).
21. Chen, A. A. et al. Humanized mice with ectopic artificial liver tissues. Proc. Natl Acad. Sci. USA 108, 11842–11847 (2011).
22. Kroon, E. et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nature Biotechnol. 26, 443–452 (2008).
23. Willenbring, H. et al. Loss of p21 permits carcinogenesis from chronically damaged liver and kidney epithelial cells despite unchecked apoptosis. Cancer Cell 14, 59–67 (2008).
Supplementary Information is available in the online version of the paper.

Acknowledgements We thank the Gladstone Institutes’ Bioinformatics Core for data analysis, A. Grimm for discussion, and P. Derish for manuscript editing. H.W. is supported by funding from the California Institute for Regenerative Medicine (CIRM; RN2-00950) and the National Institutes of Health (NIH; P30 DK26743). S.D. is supported by funding from CIRM, NIH and the Gladstone Institutes. S.Z. is supported by CIRM research training grant TG2-01160. M.R. is a research fellow in the Biomedical Exchange Program funded by the German Academic Exchange Service. J.H. is an Ethicon-Society of University Surgeons Fellow. A.N.M. is supported by CIRM research training grant TG2-01153.

Author Contributions S.Z., M.R. and J.H. are joint first authors; H.W. and S.D. are joint senior authors. S.Z., M.R., J.H., H.W. and S.D. designed the experiments. S.Z. and A.N.M. performed the reprogramming and directed differentiation experiments. M.R. and J.H. performed the transplantation experiments. M.R., J.H. and A.N.M. analysed the transplantation experiments and performed additional *in vitro* analyses. A.R.W. and L.Z.B. performed the liquid chromatography–tandem mass spectrometry analyses. S.Z., M.R., J.H., H.W. and S.D. wrote the manuscript. H.W. and S.D. edited the manuscript. All authors read and approved the final manuscript.

Author Information Results of the microarray analysis have been deposited in the Gene Expression Omnibus database under accession no. GSE52309. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to H.W. (willenbringh@stemcell.ucsf.edu) or S.D. (sheng.ding@gladstone.ucsf.edu).
METHODS

Generation of iMPC-ECs from human fibroblasts. Human newborn fibroblasts (CRL-2097; ATCC) were cultured in a 10-cm tissue culture dish coated with 0.1% gelatin, and cultured for 7, 10 or 14 days in RIM supplemented with 20 ng ml⁻¹ BFGF, 20 ng ml⁻¹ BMP4, 0.1 mM Dex and 0.5 mM A83, and finally for a further 10 days in HMM, consisting of HCM supplemented with 0.5 mM A83, 0.1 mM Dex, 20 ng ml⁻¹ HGF, 20 ng ml⁻¹ OSM and 0.1 mM C-E. Primary antibodies were goat anti-SOX17 antibody (catalogue no. AF1924; R&D) diluted 1:1,000, rabbit anti-FOXA2 antibody (catalogue no. AB4125; Millipore) diluted 1:500, goat anti-FOXA2 antibody (catalogue no. AF2400; R&D) diluted 1:1,000, mouse anti-HNF4α antibody (catalogue no. PH-H1415-00; Perseus Proteomics) diluted 1:500, mouse anti-AAT antibody (catalogue no. MA194038; Thermo Scientific) diluted 1:500, goat anti-PDX1 antibody (catalogue no. AF2419; R&D) diluted 1:1,000, goat anti-OCT4 antibody (catalogue no. sc-8629; Santa Cruz Biotechnology) diluted 1:500, rabbit anti-NANOG antibody (catalogue no. ab80892; Abcam) diluted 1:500, mouse anti-AFP antibody (catalogue no. A8452; Sigma-Aldrich) diluted 1:500, mouse anti-CK18 antibody (catalogue no. ab82254; Abcam) diluted 1:500, and goat anti-ALB antibody (catalogue no. A80-129A; Bethyl) diluted 1:500. Secondary antibodies were Alexa Fluor 488/555 donkey anti-mouse or anti-rabbit or anti-goat IgG (diluted 1:1,000) (Invitrogen). Nuclei were revealed by staining with Hoechst 33258 (Sigma-Aldrich). Images were captured with a Nikon Eclipse TE2000-U microscope.

Flow cytometry. Cells were harvested by dissociation for 2–5 min with Accutase at 37 °C, and fixed for 10 min with 4% formaldehyde in H-PBS (Sigma-Aldrich) on ice. Afterwards, cells were washed five times with ice-cold Perm/Wash buffer (BD). To remove undissociated cell clusters, cells were passed twice through 70-μm cell strainers (BD). Cells were immunostained on ice for 2 h with goat anti-FOXA2 antibody (catalogue no. AF2400; R&D) diluted 1:100, rabbit anti-NANOG antibody (catalogue no. ab80892; Abcam) diluted 1:20, mouse anti-HNF4α antibody (catalogue no. PH-H1415-00; Perseus Proteomics) diluted 1:100, goat anti-ALB antibody (catalogue no. A80-129A; Bethyl) diluted 1:100, mouse anti-CK18 antibody (catalogue no. ab82254; Abcam) diluted 1:100, or phycocyanin-conjugated mouse anti-TRA-1-60 antibody (catalogue no. 330609; Biologend) diluted 1:50. To determine background levels of each immunostaining, cell aliquots were incubated with the respective isotype control antibodies. After immunostaining, cells were washed five times with Perm/Wash buffer. Cells were then incubated on ice for 1 h with Alexa Fluor 488-conjugated or Alexa Fluor 555-conjugated secondary antibodies (Invitrogen) diluted 1:500. Afterwards, cells were washed five times with Perm/Wash buffer. Finally, cells were resuspended in 0.5 ml of ice-cold D-PBS supplemented with 2% FBS, and flow cytometry was performed on a FACS Calibur system using CellQuest software (BD). FlowJo software (Tree Star) was used to analyse the data.

qRT–PCR. Total RNA was extracted using the miRNeasy Mini Kit (Qiagen) or Direct-zap Mini Kit in combination with QuickAmp (Qiagen). First-strand reverse transcription was performed with 0.5–1.0 μg of RNA using the iScript cDNA Synthesis Kit (Bio-Rad) or qScript cDNA Supermix (Quanta Biosciences). qRT–PCR was performed with Perfecta SYBR Green SuperMix (Quanta) or iQ SYBR Green Supermix (Bio-Rad) on an Applied Biosciences Viia 7 Real-Time PCR System (Innovoitit). aHeps purchased from Yecuris or the Liver Tissue Cell Distribution System (NIH) and iHeps obtained from StemExpress were shipped overnight in suspension, centrifuged for 5 min at 300g immediately after arrival, and stored as cell pellets at −80 °C before RNA extraction. Primer sequences are shown in Supplementary Table 3. Human gene-specific primers were derived from previously published publications. CYP450 activity analysis. Luminescence-based P450-Glo Assays (Promega) were used to measure the activities of the CYP3A family (Lumifactor-PFBE, catalogue no. V9001), CYP3A4 (Lumicolor-IAPA, catalogue no. V9001) and CYP2C19 (Lumicfrin-HEG, catalogue no. V8881) in accordance with the manufacturer’s instructions. Results are shown as LUC min⁻¹ normalized to a million viable cells. Cell viability was assessed by Trypan Blue Stain (Invitrogen). Metabolically well-characterized aHeps (HMCPMS lot no. Hu8138; Life Technologies) were used as a positive control. Periodic acid–Schiff staining. Periodic acid–Schiff (Sigma-Aldrich) staining was performed in accordance with the manufacturer’s instructions.

Lipid staining. Bodipy 493/503 (4,4-difluoro-5,7-di-18-carboxyfluorescein diacetate–dioleoyl sodium salt) solution (1 mg ml⁻¹) was added to the medium (2 μl per well of a 12-well plate); 1 h later, the cells were washed with medium and imaged by fluorescence microscopy. For ORO staining, cells were fixed in 10% formalin, incubated with ORO staining solution (Sigma-Aldrich) for 1 h at 20 °C, washed with water and imaged by light microscopy.
Low-density lipoprotein (LDL) uptake assay. Dil-ac-LDL (Invitrogen) was added to the medium (5 μl per well of a 12-well plate); 2 h later, the cells were washed with medium and imaged by fluorescence microscopy.

Urea production. Cell culture supernatant was collected and analysed with the QuantiChrom Urea Assay Kit (BioAssay Systems) in accordance with the manufacturer’s instructions.

ALB ELISA of cell culture supernatants. The amount of ALB in cell culture supernatants was determined using a human-specific ALB ELISA kit (catalogue no. EA3201-1; Assaypro) in accordance with the manufacturer’s instructions. Cells were cultured in HMM for 24 h, and the supernatant was collected for analysis. Control aHePs (HMCPCMS lot no. Hu8138; Life Technologies) were analysed 24 h after plating.

Mice and transplantation. Procedures involving mice were approved by the Institutional Animal Care and Use Committee at the University of California San Francisco. Immune-deficient, fumarylactoacetate hydrolase (Fah)-deficient mice lacking B, T and natural killer cells due to disruption of Rag2 and H2zg—so-called FRG mice—were used as recipients. Mice were maintained on NTBC (Yecuris) in the drinking water at 16 mg l⁻¹. One day before transplantation, mice were taken off NTBC. An adenovirus expressing urokinase plasminogen activator (Ad–uPA) was used for liver preconditioning. Ad–uPA was delivered by retro-orbital injection 24 h before transplantation at a dose of 5 × 10⁹ plaque-forming units (PFU) per g body weight. Transplantation was performed by intrasplenic injection through a left flank incision under isoflurane anaesthesia and buprenorphine analgesia. After transplantation, mice received NTBC in the drinking water in cycles consisting of 7–10 days with NTBC absent and 2–3 days with NTBC present at 4 mg l⁻¹. For surgical prophylaxis, 5 mg of Naxcel (Pfizer) was given by intraperitoneal injection immediately before transplantation, and daily for 7 days. Because of the immune deficiency of the mice, all received additional prophylactic antibiotic treatment with Ciprofloxacin (Hospira) at 0.25 mg ml⁻¹ in the drinking water for 7 days, then trimethoprim/sulfamethoxazole (TMP/SMX, Sigma–Aldrich) in the drinking water at 0.2 g l⁻¹ TMP and 1 g l⁻¹ SMX continuously. Anti–Fah antibodies for transplantation were purchased from Yecuris, shipped overnight in suspension and transplanted into recipient mice immediately after arrival.

HSA ELISA. HSA levels were determined with the Human Albumin ELISA Quantitation Set (catalogue no. E80-129; Bethyl). Blood (3 μl) drawn by tail clipping was immediately diluted 1:100 in sample diluent; HSA concentration was determined by ELISA with a human-specific ALB antibody.

Tissue immunostaining. Liver tissue harvested from recipient mice was frozen immediately in optimum cutting temperature (OCT) compound (Tissue-Tek; Sakura Finetek) or fixed overnight in 4% paraformaldehyde (Sigma–Aldrich) or 10% formalin (Sigma–Aldrich) at 4 °C. Tissues for frozen sections were cryoprotected in 30% sucrose (Sigma–Aldrich) before embedding and freezing in OCT. For paraffin embedding, tissues were preserved in 70% ethanol before processing. Frozen tissues were cut with a Leica 3050S Cryostat into 5–7-μm sections, air dried, and stored at −20 °C before staining. Tissue sections were stained with rabbit anti–FAH antibody (gift from R. Tanguay) diluted 1:15,000 (ref. 35), mouse anti–FAH antibody (gift from R. Tanguay) diluted 1:15,000 (ref. 35), mouse anti–HSA antibody (gift from R. Tanguay) diluted 1:100 in sample diluent; HSA concentration was determined by ELISA with a human-specific ALB antibody.

Quantification of liver repopulation. Whole liver repopulation with transplanted iMPC–Heps was determined by measuring the area of recipient mouse liver sections composed of iMPC–Heps by ALB and FAH immunostaining relative to the total area of liver tissue. Sections were taken from six separate pieces of liver tissue from different parts of the recipient’s liver. Areas were calculated with ImageJ software (NIH). The number of iMPC–Heps per nodule was estimated by using a previously described method. In brief, the number of iMPC–Heps present in the two-dimensional section showing the widest diameter of a repopulating nodule was multiplied by a previously determined correction factor to estimate the total number of hepatocytes comprising the three-dimensional nodule.

Laser-capture microscopy (LCM) and microarray analysis. Repopulating nodules were isolated with a PALM MicroBeam IV system (Zeiss) about 9 months after transplantation. PALM RoboSoftware 4.3 SP1 was used to create LCM matrices based on ALB immunostaining of cryosections flanking a 7-μm unfixed cryosection from which nodules were isolated. Multiple nodules from a mouse were pooled to generate a sample. RNA was extracted and purified with the Arcturus Pico Pure RNA Isolation Kit (AB Biosystems). RNA quality was analysed with the use of chip-based capillary electrophoresis (Bioanalyzer; Agilent), and quantity and purity was determined with a NanoDrop spectrophotometer. A Pico V2 LCM kit was used for amplification, fragmentation and biotin labelling. Labelled complementary DNA was hybridized to GeneChip Human Gene 1.0 ST Arrays (Affymetrix). Signal intensity fluorescent images produced during Affymetrix GeneChip hybridizations were read with the Affymetrix Model 3000 Scanner and converted into GeneChip probe results files (CEL) using Command and Expression Console software (Affymetrix). Arrays were normalized for array-specific effects by Affymetrix Robust Multi–Array (RMA) normalization. Normalized array values were reported on a log₂ scale. For statistical analyses, background noise was eliminated by removing probesets for which no experimental group had an average log₂ intensity greater than 3. Linear models were fitted for each gene by using Bioconductor limma in R. Moderated t-statistics, fold change and the associated P values were calculated for each gene. Heatmaps were created by using heatmap.2 in R v2.11.0. Gene sets of hepatocyte function–related GO terms were obtained from MSigDB (http://www.broadinstitute.org/gsea/msigdb).

In vivo CYP2D6 activity analysis. Plasma samples (40 μl) were obtained by retro-orbital blood draw at 0.1 and 2 h after administration of debrisoquine (Enzo Life Sciences) at 2 mg per kg body weight in water by gavage. A standard curve was created by serial dilution of a solution with equal amounts of debrisoquine and 4-hydroxy-debrisoquine (Santa Cruz Biotechnology), using a 1:1 acetonitrile-water wash. Aliquots of standard solutions not exceeding 5 μl in size were added to 100 μl of Swiss Webster K₂EDTA mouse plasma (Bioreclamation) to create plasma standards with concentrations from 0.01 to 50 μM. All plasma samples were precipitated with four volumes of cold acetone/tert-vortex mixed for 1 min and, after standing for 30 min at −20 °C, centrifuged for 5 min at 13,000g. The supernatants separated into upper and lower phases; the smaller lower phases were used in the analyses. Debrisoquine and 4-hydroxy-debrisoquine were measured by LC–MS/MS with an API4000 MS/MS mass spectrometer (AB Sciex) with electrospray ionization in the positive-ion mode. They were detected with the transitions 176.1→134.1 and 192.1→132.1 m/z. Instrumental settings were 46 and 41 V for DP (declustering potential), and 25 and 27 V for CE (collision energy), respectively. Settings in common for both analytes were CXP (collision cell exit potential) = 8 V, EP (entrance potential) = 10 V, CAD (collision gas) = 12 lbf in⁻², IS (ion spray voltage) = 5,500 V, temperature = 600 °C, CUR (curtain gas) = 35 lbf in⁻² and GS1 (ion source nebulizer gas) = GS2 (ion source heater gas) = 50 lbf in⁻². The LC method employed a 50 mm × 4.6 mm C₁₈, 5-μm, 100-Akinet column (Phenomenex) and a binary mobile phase with A = 15% methanol/water (with 160 mg l⁻¹ NH₄Cl/CO₂, 0.1% formic acid and 0.1% acetonitrile) and B = 100% acetonitrile. No gradient was used with the same acetonitrile/water flow rate of 0.3 ml min⁻¹. The gradient used was as follows: 0–1 min, 0% B; 1–4 min, linear ramp to 100% B; 4–5 min, 100% B; 5–5.5 min, linear ramp to 0% B; 5.5–8.0 min, 0% B. Injection size was 3 μl and retentions were 29.4 min for 4-hydroxy-debrisoquine and 3.85 min for debrisoquine.

Survival studies. To model acute liver failure, FRG mice were taken off NTBC and injected with Ad–uPA (5 × 10⁹ PFU per g body weight) 1 day before transplantation. Mice were kept off NTBC, and survival was recorded daily. To model chronic liver failure, mice were also taken off NTBC and injected with Ad–uPA (5 × 10⁹ PFU per g body weight) 1 day before transplantation, but 7 days after transplantation NTBC was reinstated at a dose of 4 mg l⁻¹. From there on, mice were subjected to repeated cycles consisting of 10 days without NTBC absent and 3 days with NTBC present. Survival was recorded twice a week. For both liver failure models, 8–12-week-old FRG mice were used. Littermates were distributed between experimental and control groups. Male and female mice were distributed equally between groups. Blinding was not done. All transplanted mice were included in the analysis with the exception of mice that died within 24 h after transplantation, which was considered a complication of the surgery, because Fah deficiency is known to take longer to unfold.

Statistical analysis. The s.d. and s.e.m. were calculated from the average of at least three biological replicates unless otherwise specified. Data were compared between experimental and control groups by using Student’s t-test (unpaired, two-tailed) or the Mantel–Cox log-rank test. A P value of less than 0.05 was considered significant.

24. Lin, T. et al. A chemical platform for improved induction of human iPSCs. Nature Methods 6, 805–808 (2009).
25. Song, Z. et al. Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. Cell Res. 19, 1233–1242 (2009).
26. Maherali, N. et al. A high-efficiency system for the generation and study of human induced pluripotent stem cells. *Cell Stem Cell* **3**, 340–345 (2008).
27. Andreou, E. R. & Prokipcak, R. D. Analysis of human CYP7A1 mRNA decay in HepG2 cells by reverse transcription–polymerase chain reaction. *Arch. Biochem. Biophys.* **357**, 137–146 (1998).
28. Leeder, J. S. et al. Variability of CYP3A7 expression in human fetal liver. *J. Pharmacol. Exp. Ther.* **314**, 626–635 (2005).
29. Zhang, X., Ding, L. & Sandford, A. J. Selection of reference genes for gene expression studies in human neutrophils by real-time PCR. *BMC Mol. Biol.* **6**, 4 (2005).
30. Aninat, C. et al. Expression of cytochromes P450, conjugating enzymes and nuclear receptors in human hepatoma HepaRG cells. *Drug Metab. Dispos.* **34**, 75–83 (2006).
31. Martinez-Jimenez, C. P., Castell, J. V., Gomez-Lechon, M. J. & Jover, R. Transcriptional activation of CYP2C9, CYP1A1 and CYP1A2 by hepatocyte nuclear factor 4α requires coactivators peroxisomal proliferator activated receptor-γ coactivator 1α and steroid receptor coactivator 1. *Mol. Pharmacol.* **70**, 1681–1692 (2006).
32. Xie, C. Q. et al. Expression profiling of nuclear receptors in human and mouse embryonic stem cells. *Mol. Endocrinol.* **23**, 724–733 (2009).
33. Mwinyi, J. et al. Regulation of CYP2C19 expression by estrogen receptor α: implications for estrogen-dependent inhibition of drug metabolism. *Mol. Pharmacol.* **78**, 886–894 (2010).
34. Lieber, A. et al. Adenovirus-mediated urokinase gene transfer induces liver regeneration and allows for efficient retrovirus transduction of hepatocytes in vivo. *Proc. Natl Acad. Sci. USA* **92**, 6210–6214 (1995).
35. Espejel, S. et al. Induced pluripotent stem cell-derived hepatocytes have the functional and proliferative capabilities needed for liver regeneration in mice. *J. Clin. Invest.* **120**, 3120–3126 (2010).
36. Hasegawa, M. et al. The reconstituted ‘humanized liver’ in TK-NOG mice is mature and functional. *Biochem. Biophys. Res. Commun.* **405**, 405–410 (2011).
37. Wang, X. et al. Kinetics of liver repopulation after bone marrow transplantation. *Am. J. Pathol.* **161**, 565–574 (2002).
Extended Data Figure 1 | Reprogramming of human fibroblasts into endoderm progenitor cells without activation of pluripotency markers.

a, qRT–PCR shows expression levels of the endoderm-specific genes SOX17 and FOXA2 during the reprogramming process (combination of initiation and reprogramming steps of the protocol) relative to starting cells at day 0. Results are means and s.e.m. for biological replicates (n = 3).

b, Immunostainings show co-expression of SOX17 and FOXA2 in colonies at day 28. Scale bars, 100 μm.

c, Immunostainings show absence of SOX17 and FOXA2 and the pluripotency-specific markers OCT4 and NANOG in parental fibroblasts. Scale bars, 100 μm.

d, Small molecules increase the number of colonies positive in FOXA2 immunostaining at day 28. Medium containing activin A was additionally supplemented with the indicated small molecules. Results are means and s.e.m. for biological replicates (n = 3).

e, qRT–PCR shows absence of endogenous (endo) OCT4 and NANOG gene expression during reprogramming to endoderm. Gene expression levels are shown relative to ESCs. Results are means and s.e.m. for biological replicates (n = 3).

f, Flow cytometry shows absence of cells expressing the pluripotency marker TRA-1-60 at the end of the reprogramming process. Cells at day 0 and ESCs were used as controls. At least 10,000 events were collected.

g, Flow cytometry for TRA-1-60 and NANOG of 10,000 cells from a culture of human fibroblasts transduced with retroviruses expressing OCT4, SOX2 and KLF4 and grown under iPSC reprogramming conditions for 30 days shows that both markers are effective in delineating rare cells reprogrammed to pluripotency. Because the number of NANOG-positive cells is higher than the number of TRA-1-60-positive cells, and virtually all TRA-1-60-positive cells are NANOG positive, NANOG seems to be a more sensitive marker in this process. All results were replicated in at least three independent experiments.
Extended Data Figure 2 | Analysis of FOXA2 and NANOG expression at the colony and single-cell level during human fibroblast-to-iMPC-EPC reprogramming. 

**a.** Diagram showing time points of analysis.

**b.** Quantification of FOXA2-positive (red) and NANOG-positive (blue) colonies forming during the reprogramming process. Results are means ± s.e.m. for biological replicates (n = 3).

**c.** Representative immunostainings show FOXA2-positive colonies emerging at day 16 of the reprogramming process and absence of NANOG-positive colonies or cells at all time points. Scale bars, 100 μm.

**d.** Flow cytometry shows a gradual increase in the number of FOXA2-positive cells beginning at day 16 of the reprogramming process, whereas NANOG-positive cells are absent at all time points. Parental fibroblasts, ESCs and iMPC-EPCs were used as controls. At least 10,000 events were collected. All results were replicated in at least three independent experiments.
Extended Data Figure 3 | Reprogramming of human fibroblasts into iMPC-EPCs occurs earlier and is more efficient than reprogramming into iPSCs.

a, Diagram showing duration of treatment with doxycycline and time allowed for reprogramming to occur until analysis. b, Quantification of iMPC-EPC and iPSC colonies forming from human fibroblasts cultured under iMPC-EPC and iPSC reprogramming conditions, respectively, in response to different durations of treatment with doxycycline. iMPC-EPC and iPSC colonies were identified by immunostaining with FOXA2 and NANOG, respectively. Results are means and s.e.m. for biological replicates (n = 3). All results were replicated in at least three independent experiments.
Extended Data Figure 4 | Expansion and further characterization of iMPC-EPCs. a, Medium containing both CHIR and A83 promotes iMPC-EPC colony expansion. Cells treated with carrier dimethylsulphoxide (DMSO) were used as a control. Scale bars, 100 μm. b, Supplementing medium containing both CHIR and A83 with EGF and bFGF further increases the number of iMPC-EPC colonies forming after passaging. Results are means and s.e.m. for biological replicates ($n = 3$). c, Immunostainings show that expanded (passage 7) iMPC-EPCs remain positive for FOXA2 and negative for NANOG. ESCs were used as a control. Scale bars, 100 μm. d, Immunostainings show HNF4α expression in an iMPC-EPC colony after expansion (passage 4) but not at day 21 of the reprogramming process, indicating that expansion induces HNF4α expression. Scale bars, 100 μm. e, Immunostaining shows that iMPC-EPCs acquire expression of the hepatic differentiation marker AFP after exposure to bFGF and BMP4 for 4 days. f, Immunostaining shows that iMPC-EPCs acquire expression of the pancreatic differentiation marker PDX1 after exposure to retinoic acid, GDC-0449 (Sonic Hedgehog inhibitor) and LDN-193189 (BMP inhibitor) for 4 days. Scale bars, 100 μm. All results were replicated in at least three independent experiments.
Extended Data Figure 5 | Directed differentiation of iMPC-EPCs into iMPC-Heps. a, Immunostainings show that almost all iMPC-EPCs express AFP after sequential exposure to bFGF, BMP4, Dex, HGF and OSM, whereas only a subset of the cells acquire expression of ALB and AAT. Scale bars, 100 μm. b, qRT–PCR at day 18 of the hepatocyte specification step of the protocol shows an additive effect of A83 and C-E in inducing ALB gene expression. Gene expression levels are shown relative to those of iMPC-EPCs treated with DMSO. Results are means and s.e.m. for technical replicates (n = 3). All results were replicated in at least three independent experiments.
Extended Data Figure 6 | Analysis of hepatocyte function of iMPC-Heps in vitro. a, Periodic acid–Schiff (PAS) staining shows that iMPC-Heps contain glycogen. Adding Dil-ac-low-density lipoprotein (LDL) fluorescent substrate to the culture medium shows that iMPC-Heps take up LDL. Incubation with BODIPY 493/503 or staining with Oil-red-O (ORO) shows storage of lipids in iMPC-Heps. Parental fibroblasts were used as a negative control. Scale bars, 100 μm. b, iMPC-Heps (red) produce urea. The concentrations of urea measured in cell culture medium at the indicated time points are shown relative to the concentrations of urea measured in fresh medium. Parental fibroblasts (blue) were used as a negative control. Results are means ± s.e.m. for biological replicates (n = 5). c, qRT–PCR shows higher expression of several hepatocyte-specific genes including ALB and SERPINA1, and lower expression of AFP, a marker of immature hepatocytes, in iMPC-Heps than in iPSC-Heps generated using current standard protocols. Gene expression of many CYP450 enzymes is also higher in iMPC-Heps than in iPSC-Heps, indicating that iMPC-Heps have a more mature hepatocyte phenotype than iPSC-Heps. Gene expression levels in iPSC-Heps were set to 1. Results are means and s.e.m. for technical replicates (n = 3). d, iMPC-Heps secrete more ALB and have higher CYP3A family, CYP3A4 and CYP2C19 activities than iPSC-Heps generated with the iMPC-EPC/Hep generation protocol, referred to as iPSC-Heps (NP). Results are means and s.e.m. for biological replicates (n = 3); Student’s t-test, asterisk, P < 0.05; two asterisks, P < 0.01. All results were replicated in at least three independent experiments.
Extended Data Figure 7 | Quantification and isolation of repopulating nodules formed by transplanted iMPC-Heps. a, Immunostainings show a small and a large nodule of iMPC-Heps detected with a human-specific ALB antibody at 3 and 9 months after transplantation. Scale bars, 100 μm. b, Multiple large nodules of iMPC-Heps identified by FAH immunostaining at 9 months after transplantation. Scale bar = 100 μm. c, Size distribution of nodules of iMPC-Heps 9 months after transplantation based on ALB and FAH immunostaining. d, Example of an iMPC-Hep nodule identified by ALB immunostaining for isolation by laser-capture microscopy (LCM). Blood vessels (numbers) were used as additional markers of the location of a nodule in an adjacent, unfixed cryosection. e, Confirmation of successful isolation of an iMPC-Hep nodule by ALB immunostaining after LCM. The middle image shows a cryosection fixed and immunostained for ALB after LCM to confirm specific isolation of a nodule. The left and right images show ALB immunostainings of cryosections flanking the cryosection used for LCM. Scale bars, 100 μm. All results were replicated in at least three independent experiments.
Extended Data Figure 8 | Assessment of in vivo maturation of iMPC-Heps by global gene expression profiling. a, Venn diagram showing the number of genes significantly (P < 0.05) differentially expressed between iMPC-Heps and aHeps in vivo. Of 17,367 reliably detected genes, 132 are differentially expressed; 78 are expressed higher in iMPC-Heps, and 54 are expressed higher in aHeps. The complete results of the global gene expression profiling—including the genes that are differentially expressed between aHeps and iMPC-Heps in vivo—are shown in Supplementary Table 2. b–e, Further analysis of results from global gene expression profiling using gene sets of the hepatocyte function-related Gene Ontology (GO) terms REACTOME CYTOCHROME P450 ARRANGED BY SUBSTRATE TYPE (b), BILE ACID METABOLIC PROCESS (c), GLUCOSE METABOLIC PROCESS (d) and RESPONSE TO XENOBIOTIC STIMULUS (e). GO terms and annotated genes were obtained from Molecular Signatures Database (MSigDB) v.4.0. Heatmaps were generated individually for each GO term; a representative colour legend is shown. All results are from one microarray analysis.
Extended Data Figure 9 | Assessment of in vivo maturation of iMPC-Heps by immunostaining. a, Co-immunostaining for ALB and AFP shows lack of expression of the immature hepatocyte-specific marker AFP in iMPC-Hep and aHep nodules. Human fetal liver was used as a positive control. Scale bars, 100 μm. b, c, Co-immunostainings for ALB and CYP3A4 (b) or CYP2D6 (c) show expression of these mature hepatocyte-specific markers in iMPC-Heps. The CYP450 antibodies detect the mouse homologues of CYP3A4 and CYP2D6, which—as in humans—seem to be expressed in hepatocytes but not in non-parenchymal liver cells. Scale bars, 100 μm. All results were replicated in at least two independent experiments.
Extended Data Figure 10 | Therapeutic efficacy and safety of iMPC-Heps.

a, Kaplan–Meier survival curve shows that $10^6$ transplanted iMPC-Heps, iPSC/ESC-Heps or aHeps are not effective in rescuing mice from death from acute liver failure. Log-rank test $P = 0.4426$ between iMPC-Heps and iPSC/ESC-Heps, $P = 0.4031$ between iMPC-Heps and aHeps. 

b, Kaplan–Meier survival curve shows similar efficacy of $10^6$ transplanted aHeps and iMPC-Heps, but not iPSC/ESC-Heps, in preventing death in mice with chronic liver failure. Log-rank test $P < 0.01$ between iMPC-Heps and iPSC/ESC-Heps, $P = 0.9501$ between iMPC-Heps and aHeps. The number of mice in each group is shown in parentheses.

c, Haematoxylin/eosin staining shows a dysplastic nodule in the liver of an FRG mouse transplanted with iMPC-Heps. Scale bar, 100 μm.

d, Co-immunostaining with human-specific β2-microglobulin (B2M) and ALB antibodies shows that the cells within a dysplastic nodule (dashed line) are negative for both markers and are therefore of mouse origin. Scale bars, 100 μm. Nodules of iMPC-Heps or aHeps are shown as controls. All results were replicated in at least two independent experiments.