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Supplementary Materials for

In situ expansion of engineered human liver tissue in a mouse model of chronic liver disease

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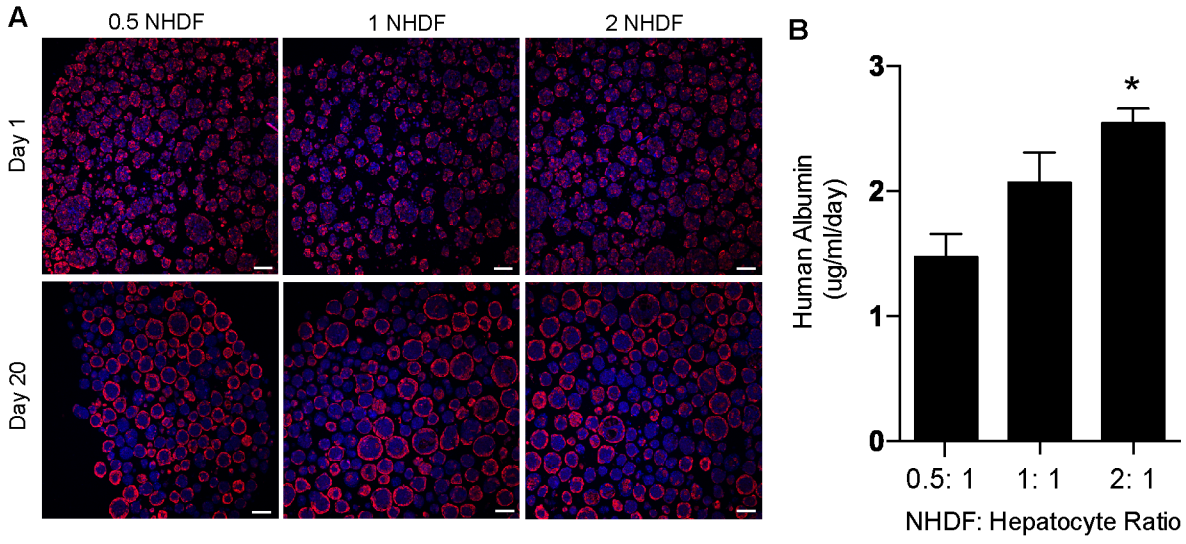
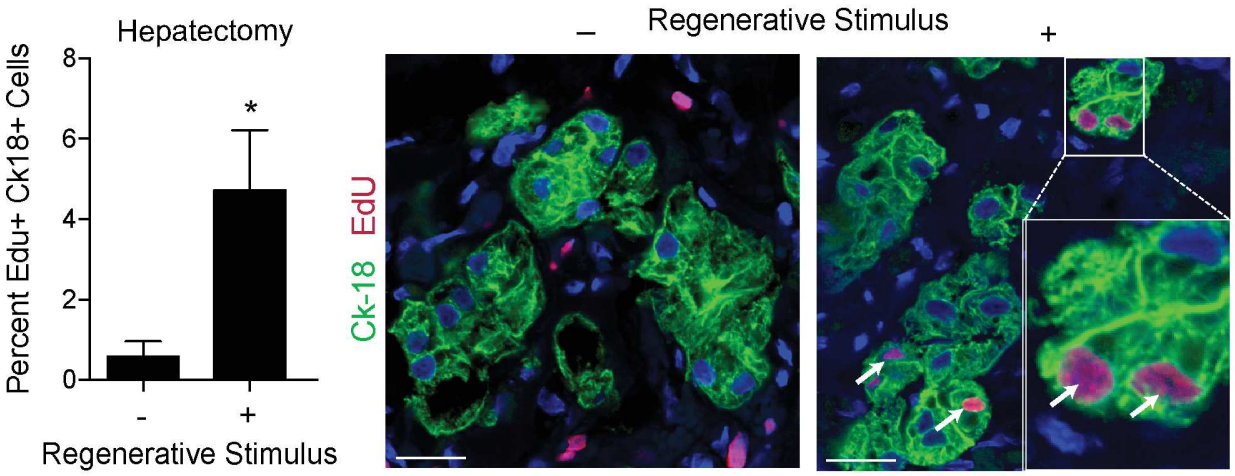
Supplemental Figures

Supplemental Figure 1: Bioprinting scalable liver tissue seeds. Sacrificial lattices of carbohydrate glass were constructed using a custom built 3D printer containing a heated nozzle, (left), embedded within a fibrin hydrogel, and dissolved using PBS to leave open channels (center). Channels were filled by pipetting a slurry containing endothelial cells (HUVECs) and neutralized collagen, and tissues were then either fixed and stained with hematoxylin (center, inset) or stained with a 'live cell' dye, calcein, and imaged via live microscopy (right).

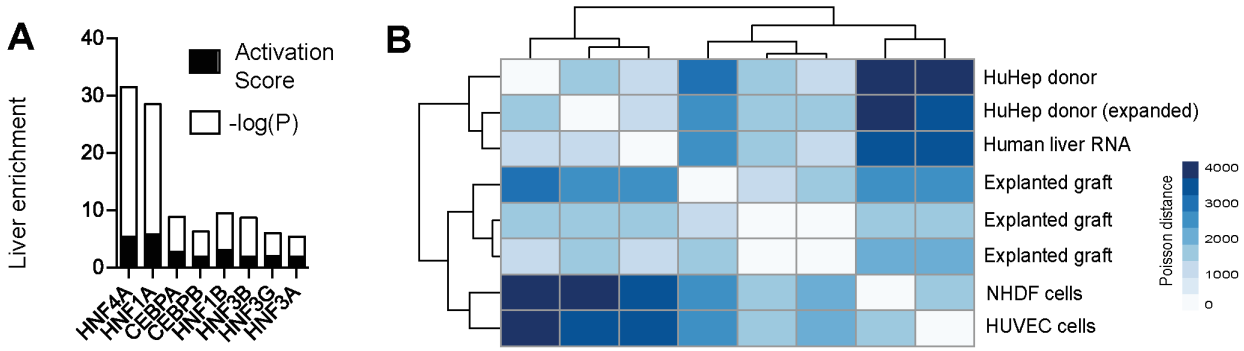


Supplemental Figure 2: Hepatic aggregates formed from hepatocyte and fibroblasts. Hepatic aggregates were created from primary human hepatocytes and normal human dermal

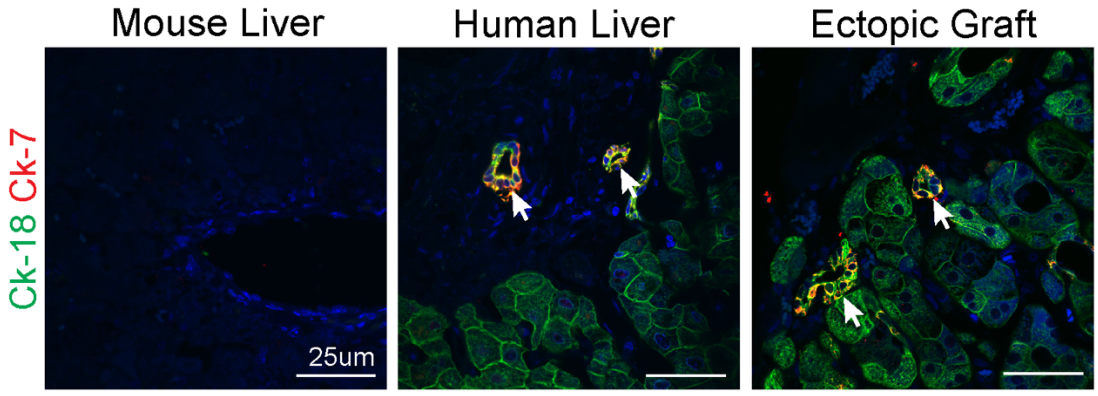
fibroblasts (NHDFs) in varying ratios. Hepatocytes and NHDFs in aggregates self-organized over 20 days *in vitro* (A). The addition of NHDFs resulted in a dose-dependent increase in cumulative albumin production (B). *P < 0.05, one way ANOVA (followed by post-hoc test). Scale bars 100 µm.



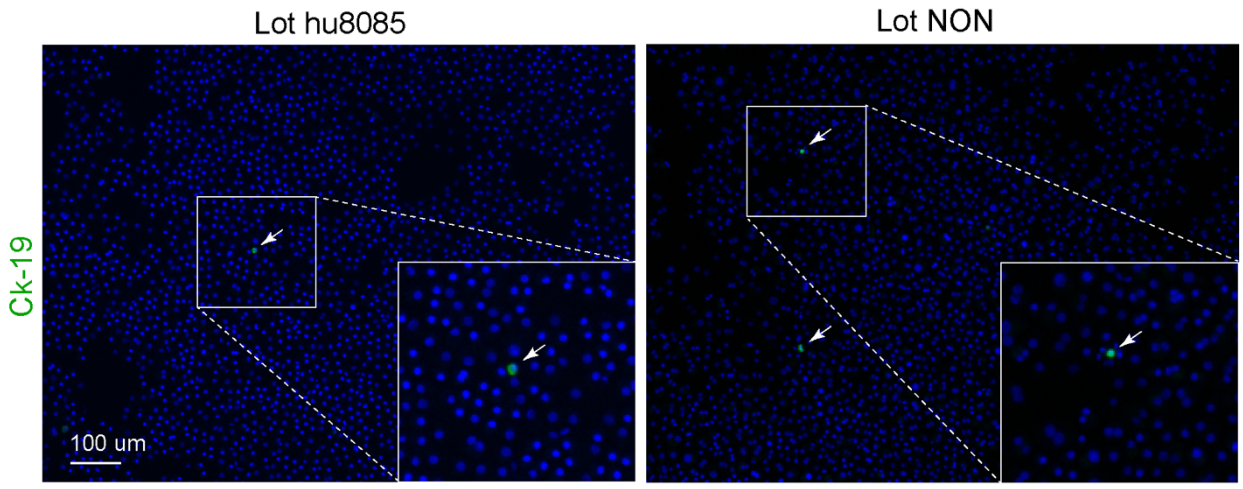
Supplemental Figure 3: Liver seed grafts synthesize DNA after partial hepatectomy. Liver tissue seeds were implanted in the mesenteric fat of athymic mice. Partial hepatectomy was performed one week following SEEDs implantation, and animals were pulsed daily with EdU to mark cells in the S-phase of the cell cycle. After 7 days, liver seed grafts in animals with hepatectomy (+ regenerative stimulus) contained significantly more Ck-18 and EdU double-positive hepatocytes compared to controls (- regenerative stimulus). * p < 0.05. Scale bars 25 µm.



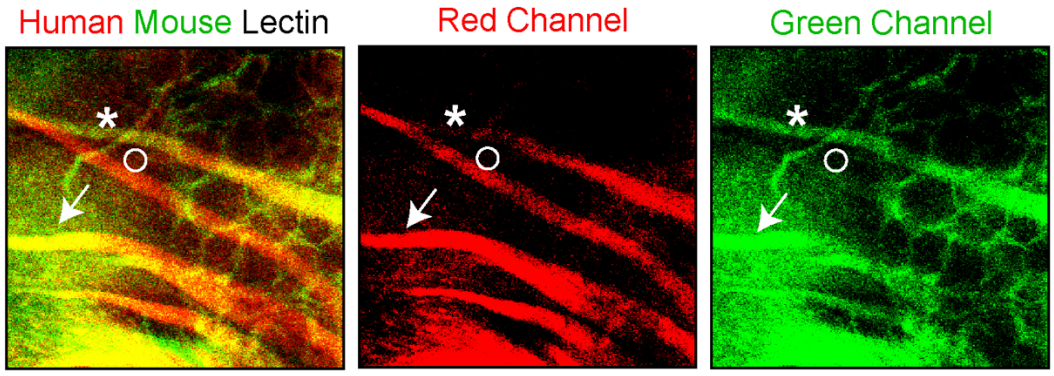
Supplemental Figure 4: Global trancriptome of liver seed grafts. Ingenuity Pathway Analysis identified regulation of transcription in liver seed grafts by hepatocyte-transcription factors, as assessed by the fraction of genes downstream of transcription factors that were differentially regulated between expanded liver seed grafts and HUVEC/NHDF controls (A). Hierarchical clustering of transcriptomes identified by RNA-seq of expanded liver seed grafts, pure human primary hepatocytes, health adult human liver, and pure populations of NHDFs and HUVECs demonstrated that liver seed grafts cluster between the primary hepatocyte/human liver samples and non-parenchymal HUVEC/NHDF cell lines (B).



Supplemental Figure 5: Liver seed grafts contain cells that express biliary epithelial cell markers. Untransplanted control mouse livers are negative for both human-specific Cytokeratin-18 (Ck-18) and Cytokeratin-7 (Ck-7) (left). Commercially available positive control human adult liver sections demonstrate that hepatocytes stain positively for Ck-18 but not Ck-7, and bile ducts stain positively for both Ck-18 and Ck-7 (center). Ectopic liver seed grafts explanted after 80 days in an NTBC-cycled mouse contain both human Ck-18-positive hepatocytes and self-assembled Ck-18 and Ck-7 double-positive structures that resemble bile ducts.

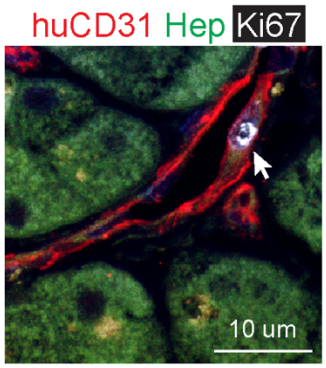


Supplemental Figure 6: Human hepatocyte samples contain rare Ck-19-positive cells. Commercially-available, cryopreserved human hepatocytes from two donors (sold as sample 'lots' designated as 'hu8085' or 'NON') were deposited on glass slides immediately after thawing using Cytospin centrifugation, and then immunostained using antibodies that recognize Cytokeratin-19 (Ck-19) (green). Rare Ck-19-positive cells were identified in both hu8085 (left) and NON (right) lots. Ck-19-positive cells accounted for 0.16% and 0.13% of total cells in lots hu8085 and NON, respectively.



Supplemental Figure 7: Liver seed grafts contain blood vessels lined with human and mouse endothelial cells. Liver tissue seeds were engrafted into FNRG animals with liver injury, and explanted liver seed grafts were then cleared using SWITCH[54] and incubated with

lectins that bind to human (red) or mouse (green) endothelium. Liver seed grafts contained vessels that were lined with both human and mouse endothelium (arrow, section of vessel containing both human and mouse endothelium; open circle, section of vessel with primarily human endothelium; asterisk, section of vessel with primarily mouse endothelium).



Supplemental Figure 8: Liver seed grafts contain rare endothelial cells that express markers associated with the cell cycle. Human liver seed grafts explanted after 80 days contained rare Ki67 and human CD31 double-positive cells (arrow).