Appendix A. Supplementary material

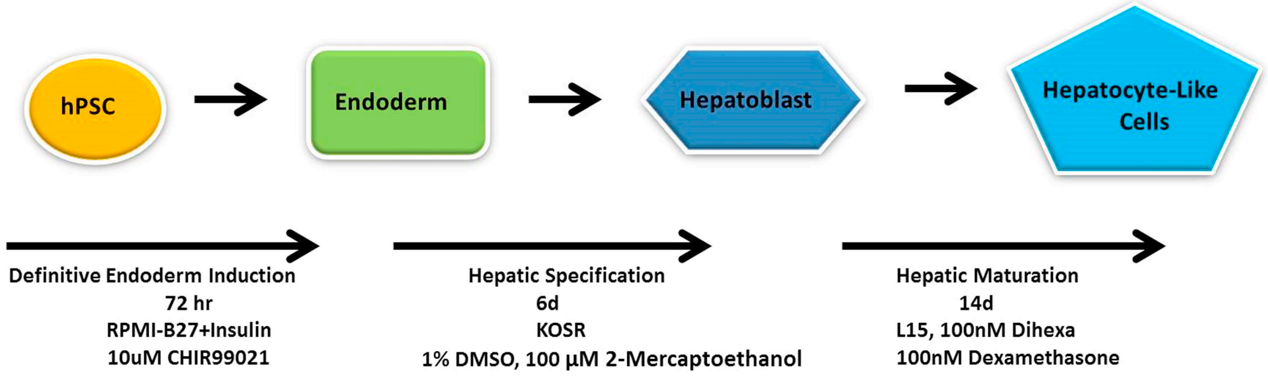


Fig. S1. Schematic of the phases of differentiation.

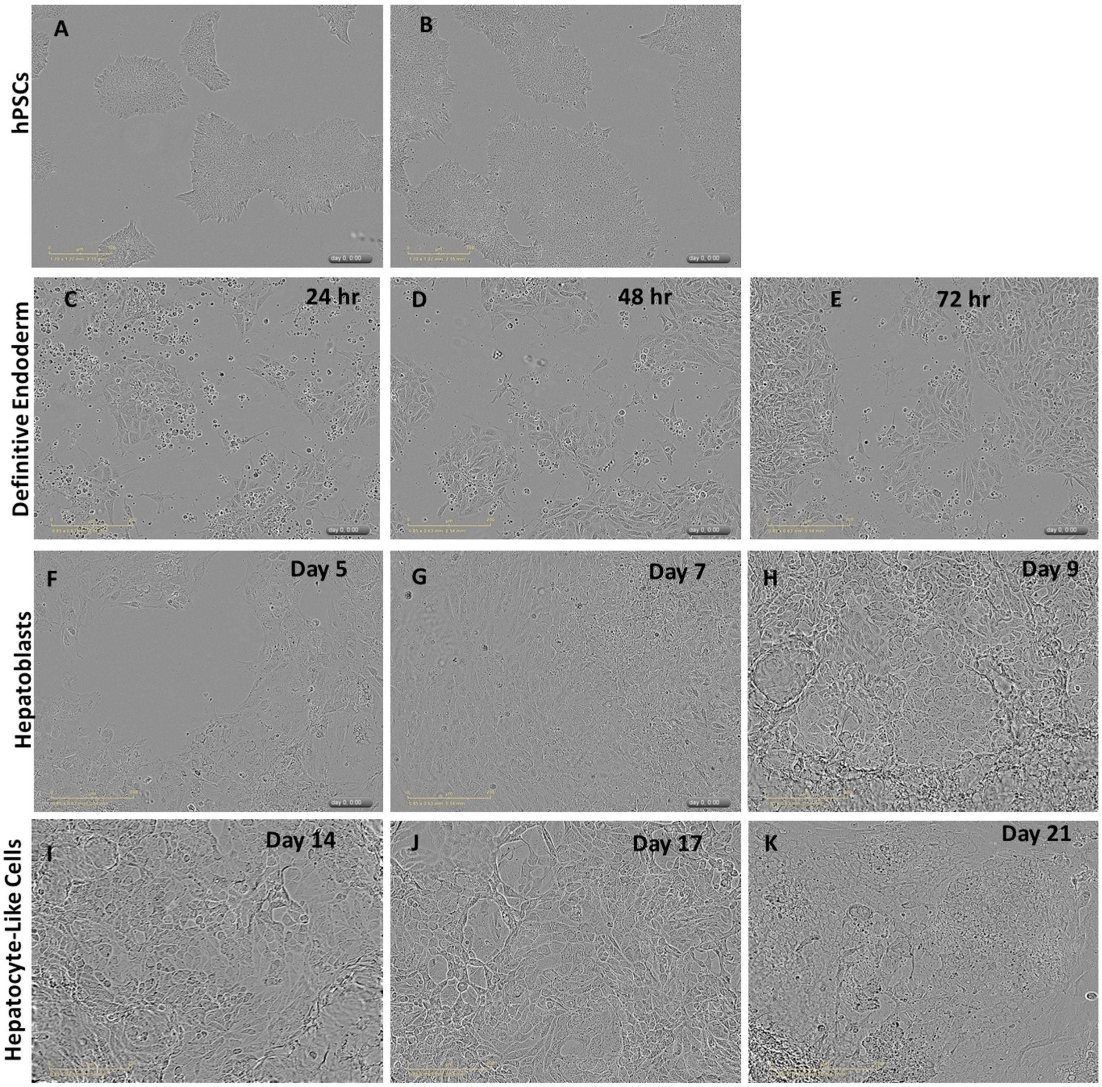
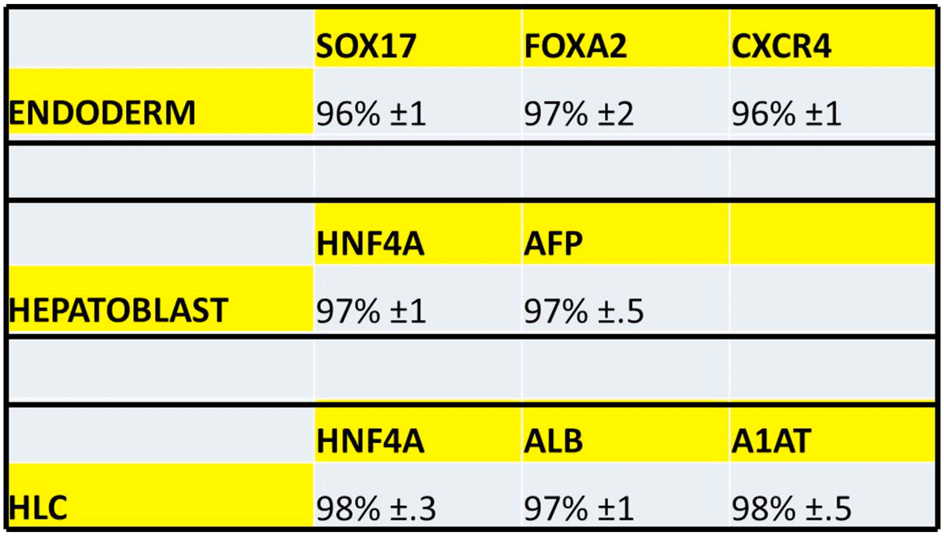


Fig. S2. **Analysis of differentiation of hPSCs to hepatocyte-like cells.** (A-B) Representative phase-contrast micrographs showing typical hPSC morphology and confluence prior to initiation of differentiation. (C-E). Time course of changes in morphology of cultured hPSCs following treatment with DE differentiation media. (F-H) Time course of changes in morphology of cultured hPSCs following treatment with hepatoblast differentiation media. (I-K) Time course of changes in morphology of cultured hPSCs following treatment with hepatic maturation media. (original magnification 20x). Representative data from three independent experiments are shown.



	SOX17	FOXA2	CXCR4
ENDODERM	96% ±1	97% ±2	96% ±1
	HNF4A	AFP	
HEPATOBLAST	97% ±1	97% ±.5	
	HNF4A	ALB	A1AT
HLC	98% ±.3	97% ±1	98% ±.5

Fig. S3. Efficiency of the different phases of differentiation (definitive endoderm, hepatic specification and hepatic maturation) as assessed by counting SOX17, FOXA2, CXCR4, HNF4A, AFP, ALB and A1AT positive cells. A minimum of 10 fields of view were quantified, with a minimum of 300 cells counted per field of view. Efficiencies are presented as a percentage of positive cells plus or minus the standard deviation across all fields counted. Representative data from three independent experiments are shown.

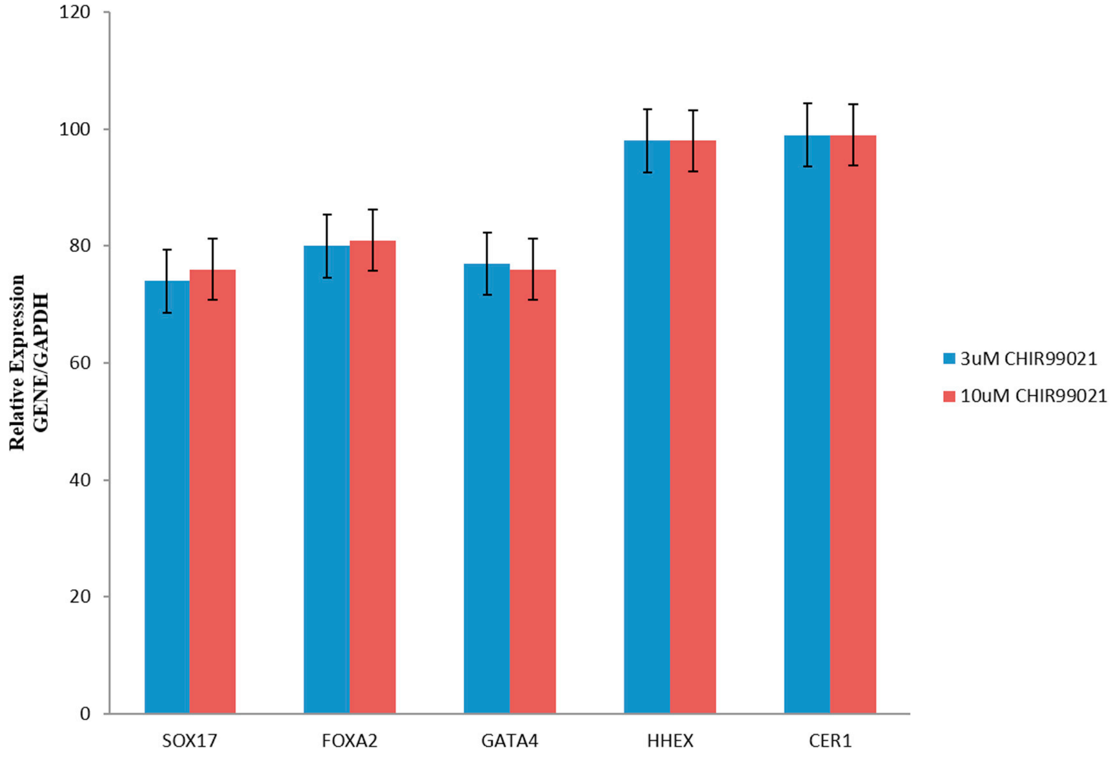


Fig. S4. Relative expression of endoderm genes SOX17, FOXA2, GATA4, HHEX and CER1 were assessed at 3uM and 10uM concentration of CHIR99021. Data represent relative expression of transcripts normalized relative to GAPDH and undifferentiated controls. Data are represented as Mean± SEM for three biologically independent experiments (n = 3).

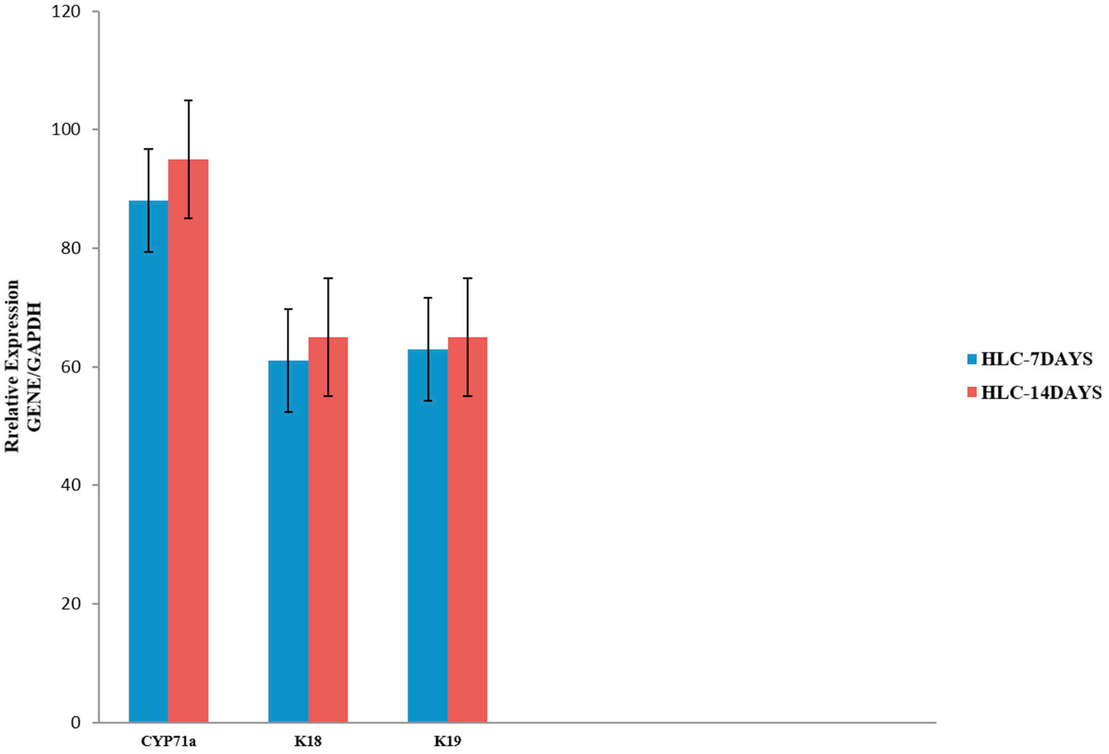


Fig. S5. Relative expression of hepatic genes CYP71a, K18 and K19 were assessed in hepatocyte-like cells. Data represent relative expression of transcripts normalized relative to GAPDH and undifferentiated controls. Data are represented as Mean± SEM for three biologically independent experiments (n = 3).