Supporting information

Robust expansion and functional maturation of human hepatoblasts by chemical strategy

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Supplementary figures

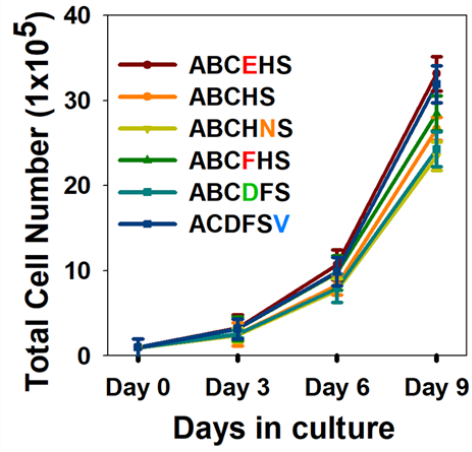

Fig. S1. Growth curve of HBs cultured in different conditions.

Cell growth curves were analyzed by obtaining a cell count. Data are presented as mean ± SEM, n = 3.

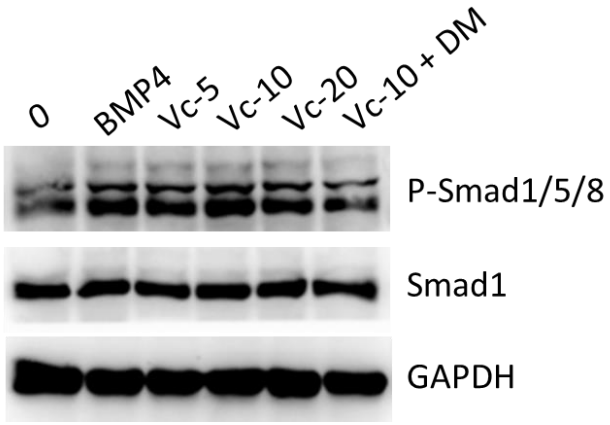

Fig. S2. Vitamin C phosphorylate Smad1/5/8 in HBs culture.

Western blot analyzed phosphorylation of Smad1/5/8 after Vc treated in HBs. Vc-5: 5 μg/mL Vc, Vc-10: 10 μg/mL Vc, Vc-20: 20 μg/mL Vc, DM: 0.5 μM Dorsomorphin.

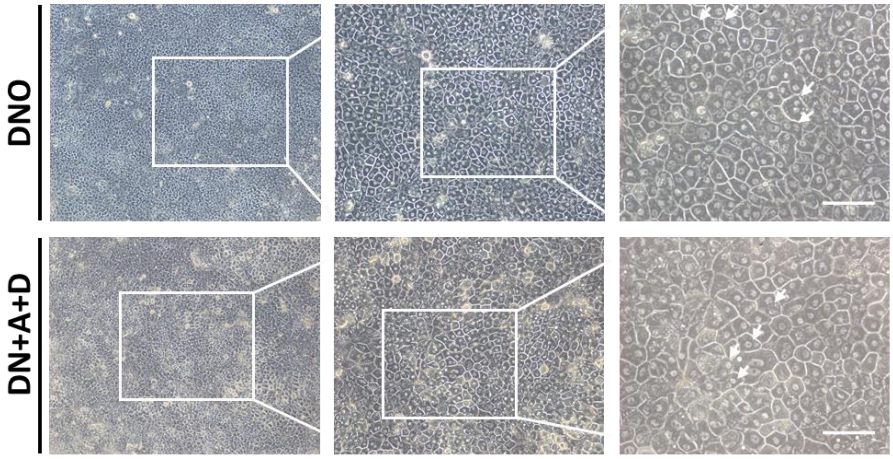

Fig. S3. The morphology of expanded HBs derived HLCs.

After 6 days of induction with hepatic maturation condition, the expanded HBs efficiently differentiated into a homogenous HLCs with typical hepatocyte morphology, with polygonal shape and distinct round nuclei even some has two nuclei (White arrows). Scale bar 100 μM.

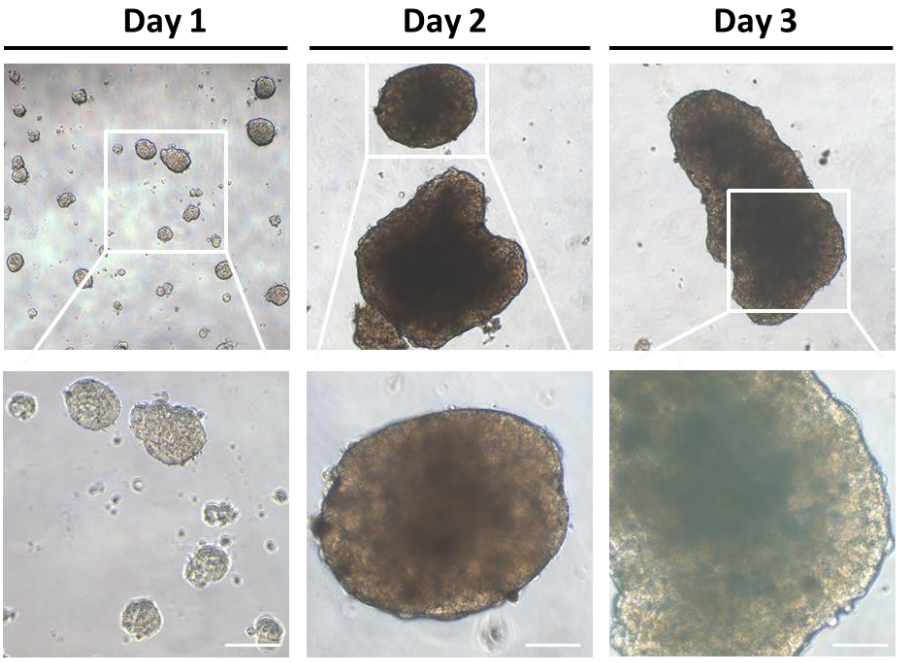

Fig. S4. Heterogeneous size of HBs spheroid in suspension culture.

HBs suspension culture in low-attachment wells produced spheroid population with heterogeneous and uncontrollable size. Scale bar 200 μM.

Supplementary Tables

Table 1. List of antibodies used in this study

Primary anbibody	Species	Company	Cat Number	Dilution
HNF4α	Rabbit	Genetex	GTX62347	1:200
AFP	Mouse	Genetex	GTX84948	1:200
E-CAD	Goat	R&D systems	AF648	1:200
Ki67	Rat	Thermo Fisher	11-5698-82	1:100
	Rabbit	Genetex	GTX16667	1:200
Ep-CAM	Mouse	Invitrogen	53-8326-42	1:100
	Mouse	Abcam	ab46714	1:100
ALB	Goat	R&D systems	MAB1455	1:200
	Rabbit	Dako	F0117	1:50
SOX9	Mouse	Abcam	ab76997	1:200
OCT4	Mouse	Genetex	GTX627423	1:200
AAT	Rabbit	Abcam	AB166610	1:200
CYP3A4	Mouse	Genetex	GTX60577	1:200
CYP2C9	Rabbit	Genetex	GTX55583	1:200
Second antibody	**Species**	**Company**	**Cat. Number**	**Dilution**
Anti-Goat IgG Antibody, Alexa Fluor 488	Donkey	Invitrogen	A-11055	1:500
Anti-Goat IgG Antibody, Alexa Fluor 568	Donkey	Invitrogen	A-11057	1:500
Anti-Mouse IgG Antibody, Alexa Fluor 488	Donkey	Invitrogen	A32766	1:500
Anti-Mouse IgG Antibody, Alexa Fluor 568	Donkey	Invitrogen	A10037	1:500
Anti-Rabbit IgG antibody Alexa Fluor 488	Donkey	Invitrogen	A-21206	1:500
Anti-Rabbit IgG antibody Alexa Fluor 568	Donkey	Invitrogen	A10042	1:500

Table 2. List of primers for real-time PCR

Gene	Primer sequence (forward / reverse; 5' to 3')
AFP	AGAACCTGTCACAAGCTGTG / GACAGCAAGCTGAGGATGTC
HNF4α	TGTACTCCTGCAGATTTAGCC / CTGTCCTCATAGCTTGACCT
CK19	ACCAAAGCTCACGCGTGGAAA / TGATGTGTCTCTCGGTCAAGTT
Ki67	GCCTGCTCGACCCTACAGA / GCTTGTCAACTGCGGTTGC
ALB	TGCAACTCTTCGTGAAACCTATG / ACATCAACCTCTGGTCTCACC
AAT	ATGCTGCCCAGAAGACAGATA / CTGAAGGCGAACTCAGCCA
ARG1	TGGACAGACTAGGAATTGGCA / CCAGTCCGTCAACATCAAAACT
ASS1	AGGAAAGGGGAACGATCAGGT / GTGTTGCTTTGCGTACTCCA

ASL	CAGTGGACCCCATCATGGAGA / GGCTTTGCTGCCTTGAACATC
CPS1	ACTTCAGTTGAGTCCATTATGGC / GGAACGGATCATCACTGGGTAG
CYP2D6	GTGTCCAACAGGAGATCGACG / CACCTCATGAATCACGGCAGT
CYP3A4	TTCAGCAAGAAGAACAAGGACAA / GGTTGAAGAAGTCCTCCTAAGC
CYP2C9	CTACAGATAGGTATTAAGGACA / GCTTCATATCCATGCAGCACCAC
CYP1A2	CTTCGTAAACCAGTGGCAGG / AGGGCTTGTTAATGGCAGTG
GAPDH	CAAAGTTGTCATGGATGACC / CCATGGAGAAGGCTGGGG