

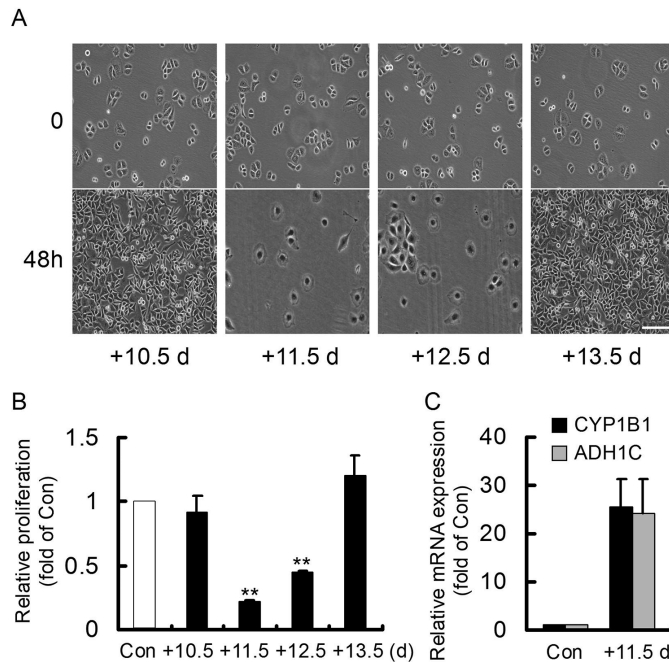
Supplemental Data

Developmental Stage-Specific Hepatocytes Induce Maturation of HepG2 Cells by Rebuilding the Regulatory Circuit

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Supplementary Figure S1. Induced maturation of SMMC-7721 cells. (A) After treated with 10.5-, 11.5-, 12.5- and 13.5-d mouse embryonic hepatocyte medium, SMMC-7721 cells were observed. The existing adherent SMMC-7721 cells became round and hexagonal in 11.5-d and 12.5-d groups, however, in 12.5-d and 15.5-d groups, obvious change was hardly observed. Scale bar = 50 μ m. (B) The proliferation of induced SMMC-7721 cells was detected by MTT, which was significantly inhibited in 11.5-d and 12.5-d groups. (C) The expression of CYP1B1 and ADH1C in SMMC-7721 cells treated with 11.5-d embryonic hepatocyte medium increased dramatically. ** $P < .01$ vs. Con (control). The experiment was repeated three times.

Supplementary Table S1. Primers used for real-time PCR detection.

	Primers	Annealing temperature	Product size
CYP3A4: Sense	5' ACGGGACTATTCCACCACC 3'	60 °C	234 bp
Anti-sense	5' TGTGCAGGAAAGCATCTGATA 3'		
CYP1B1: Sense	5' AGTGCAGGCAGAATTGGA 3'	60 °C	159 bp
Anti-sense	5' GAGGTGTTGGCAGTGGTG 3'		
OTC: Sense	5' TTTGCTCCCTCACTGCAACT 3'	60 °C	209 bp
Anti-sense	5' TGTAGTGGTTGTCCACACCG 3'		
ARG1: Sense	5' ACGGAAGAATCAGCCTGGTG 3'	60 °C	281 bp
Anti-sense	5' GTCCACGTCTCTCAAGCCAA 3'		
ADH1C: Sense	5' GCACCTCCTAAGGCTCAT 3'	60 °C	147 bp
Anti-sense	5' TCCAACACTTCCACGAT 3'		
c-Myc: Sense	5' CGGGGCTTTATCTAACTCGC 3'	60 °C	224 bp
Anti-sense	5' GCTATGGGCAAAGTTTCGTG 3'		
HNF-4 α : Sense	5' GATGACAATGAGTATGCCTACCT 3'	65 °C	132 bp
Anti-sense	5' GTCGTTGATGTAGTCTCCAA 3'		
HNF-1 α : Sense	5' TTCGTGGGATACAGTCTTCTTAC 3'	60 °C	463 bp
Anti-sense	5' ATGATGCTCTTGGGAACAAATAC 3'		
HNF-6: Sense	5' CCCTGGAGCAAACCTCAAATC 3'	62 °C	147 bp
Anti-sense	5' TGTGTGCCTCTATCCTTCC 3'		
USF-1: Sense	5' TGGCACTGGTCAATCTTTGTG 3'	60 °C	168 bp
Anti-sense	5' GTTGCTGTCAATCTTGATTGAC 3'		
β -actin: Sense	5' CTCCATCCTGGCCTCGCTGT 3'	60 °C	268 bp
Anti-sense	5' GCTGTCACCTCACCGTTCC 3'		

Supplementary Table S2. Primers used for ChIP detection.

	Primers	Annealing temperature	Product size
HNF-4 α to HNF-1 α :		65°C	196 bp
Sense	5' GTTCAGTCCCTTCGCTAAGCACACG 3'		
Anti-sense	5' CGGCAGACACAAACCAAACCTCCTTG 3'		
HNF-4 α to USF-1 proximal:		60°C	168 bp
Sense	5' AACTGGACTAAGTATCCTGTGCTCA 3'		
Anti-sense	5' ATCTCTGCCACTTCTTACCTCTGA 3'		
HNF-4 α to USF-1 distal:		60°C	360 bp
Sense	5' AAGTTTCCTGAGTGCCTATGATGA 3'		
Anti-sense	5' CAGCCTTCTCTGACCTTTGGAC 3'		
USF-1 to HNF-6:		65°C	318 bp
Sense	5' TAGAGCCGAAGGGGTGATGACACAG 3'		
Anti-sense	5' GGCCTCCCGGTACAAATGAAGGAG 3'		
HNF-6 to HNF-4 α proximal:		65°C	343 bp
Sense	5' TCGAGGCAGCCTTATCTCTGCAAAAGC 3'		
Anti-sense	5' TCGAGGGGTGGGGTAATGGTTAATCGG 3'		
HNF-6 to HNF-4 α distal:		65°C	365 bp
Sense	5' GGCTCTGACACTGCAGAGTTCTAGAAC 3'		
Anti-sense	5' CCAAACCTTACCCAGCTGCTAATCATTGC 3'		
HNF-1 α to HNF-4 α :		65°C	343 bp
Sense	5' TCGAGGCAGCCTTATCTCTGCAAAAGC 3'		
Anti-sense	5' TCGAGGGGTGGGGTAATGGTTAATCGG 3'		
NF- κ B to iNOS-5212:		60 °C	226 bp
Sense	5' AGGGAAGGGAGGGTGTCTGTTCTGGGGAG 3'		
Anti-sense	5' TTGCCTGACTCGGAGATGACGGAAG 3'		

Supplementary Table S3. The oligonucleotides for HNF-4 α sites in HNF-1 α and USF-1 promoters.

Oligonucleotides	
HNF-1 α +428	
forward	biotin-5' GCAGGGCTGAAGTCCAAAGTTCAGTCCCTTCGCTAAGCAC 3'
reverse	biotin-5' GTGCTAGCGAAGGGACTGAACCTTGGACTTCAGCCCTGC 3'
Mutant+428	
forward	biotin-5' GCAGGGCTGAAGTCCATGCGTTCAGTCCCTTCGCTAAGCAC 3'
reverse	biotin-5' GTGCTAGCGAAGGGACTGACGCATGGACTTCAGCCCTGC 3'
USF-1-2882	
forward	biotin-5' CCAAGGAAGAGAAGTCCAAAGGTCAGAAGAAGGCTGACAG 3'
reverse	biotin-5' CTGTCAGCCTTCTCTGACCTTGGACTTCTTCCCTTGG 3'
Mutant-2882	
forward	biotin-5' CCAAGGAAGAGAAGTCCATGCGTTCAGAAGAAGGCTGACAG 3'
reverse	biotin-5' CTGTCAGCCTTCTCTGACGCATGGACTTCTTCCCTTGG 3'
USF-1-612	
forward	biotin-5' GTCAAAATGTTTGCCAAAGGTCTCAGAGGTAAGAAGTGGC 3'
reverse	biotin-5' GCCACTTCTTACCTCTGAGACCTTGGCAAACATTTTGAC 3'
Mutant-612	
forward	biotin-5' GTCAAAATGTTTGCCATGCGTCTCAGAGGTAAGAAGTGGC 3'
reverse	biotin-5' GCCACTTCTTACCTCTGAGACGCATGGCAAACATTTTGAC 3'