

Title

Direct conversion of human fibroblasts into hepatocyte-like cells
by ATF5, PROX1, FOXA2, FOXA3, and HNF4A transduction

Authors

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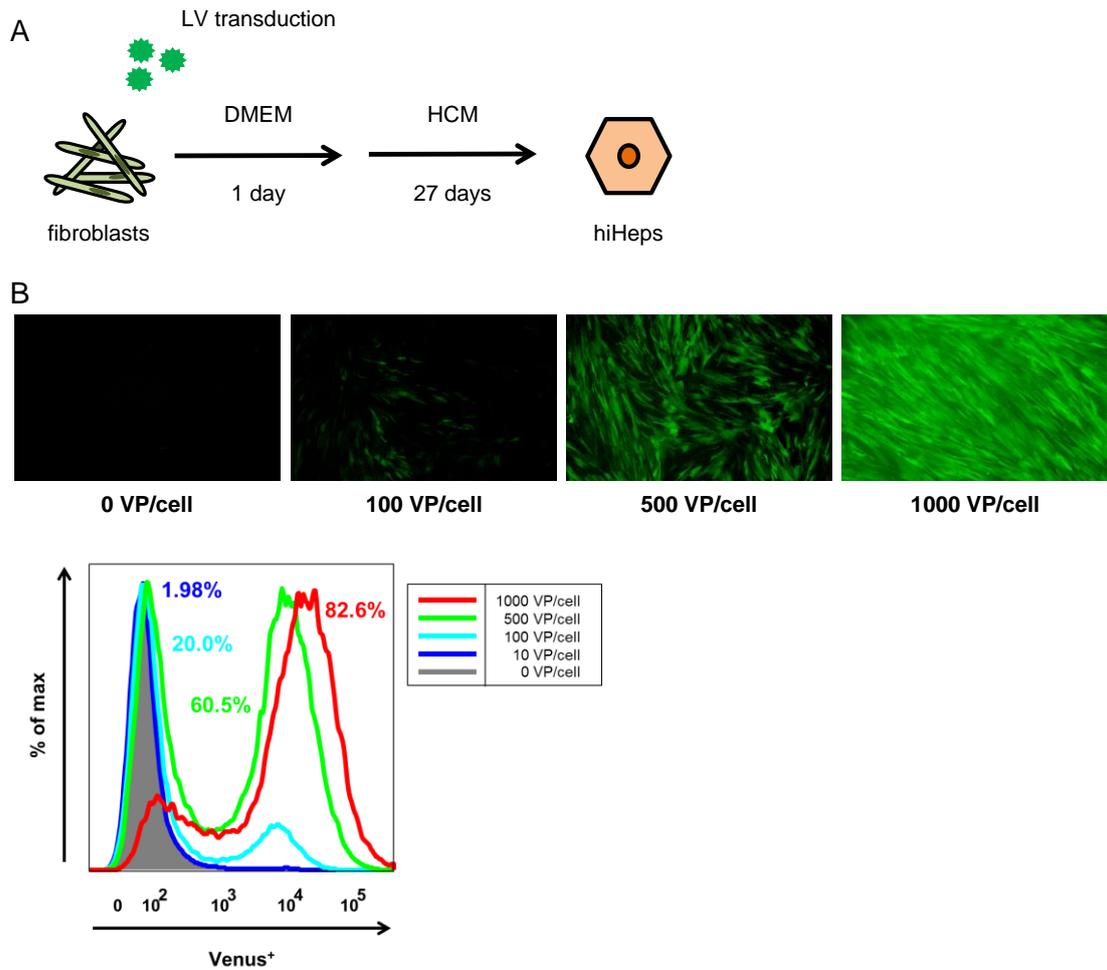


Figure S1 Induction of hepatocyte-like cells from human fetal fibroblasts

(A) The procedure for conversion of human fetal fibroblasts (MRC5) cells into hiHeps is presented schematically. MRC5 cells were transduced with hepatic transcription factor-expressing lentivirus vectors (LV-TFs) for 12 hr, and cultured until day 28. (B) MRC5 cells were transduced with 0, 100, 500, or 1,000 VP/cell of LV-Venus (modified green fluorescent protein) for 12 hr, and cultured until day 3. The percentage of Venus (green)-positive cells was examined by FACS.

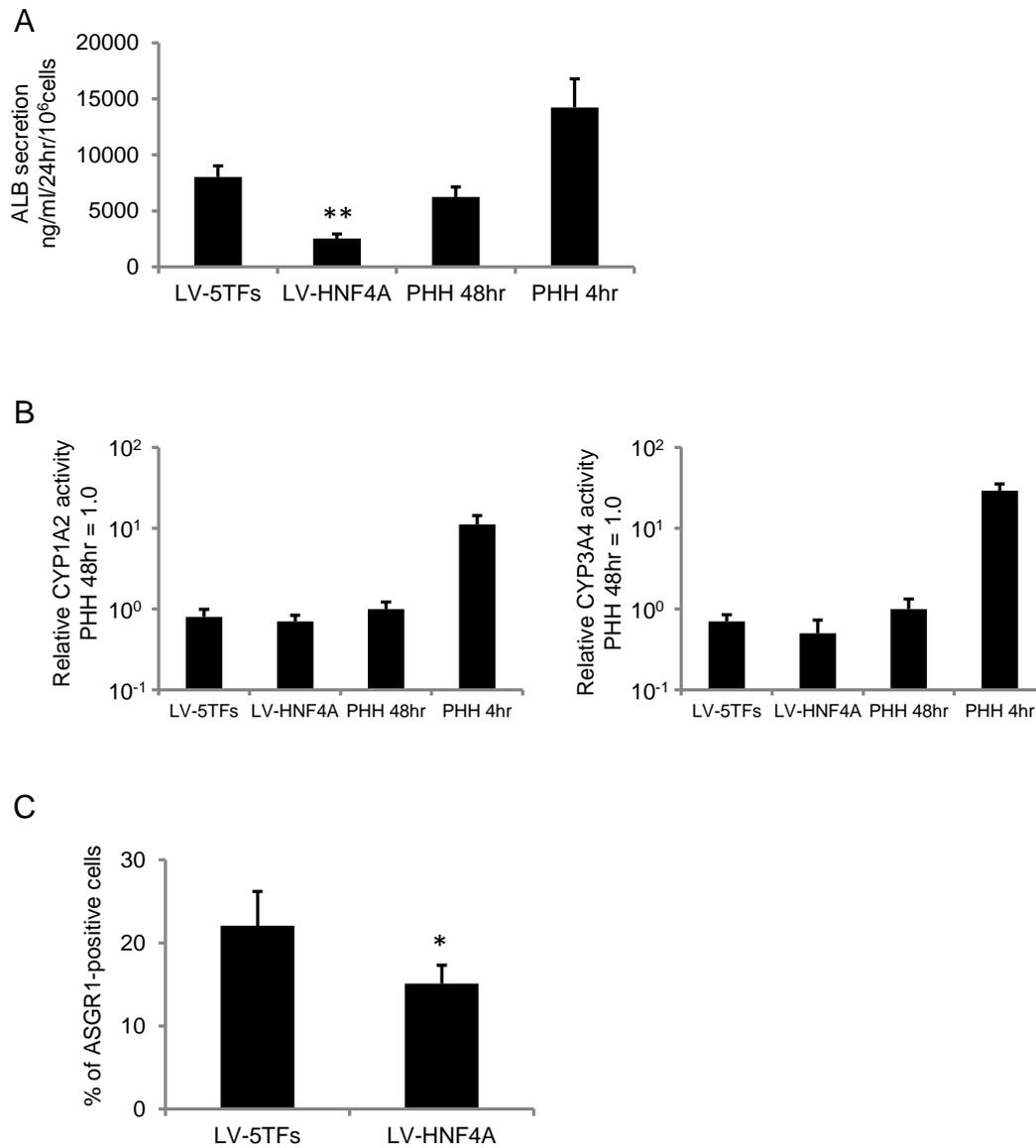


Figure S2 Generation of hiHeps by HNF4A transduction

MRC5 cells were transduced with LV-5TFs or LV-HNF4A for 12 hr, and cultured until day 28. **(A)** The ALB secretion capacity in LV-5TFs- and LV-HNF4A-transduced cells, PHH 48hr, and PHH 4hr was examined by ELISA. ****** $p < 0.01$ (vs LV-5TFs). **(B)** The CYP1A2 and CYP3A4 activities were examined in LV-5TFs- and LV-HNF4A-transduced cells, PHH 48hr, and PHH 4hr. The CYP1A2 and CYP3A4 activity levels in PHH 48hr were taken as 1.0. **(C)** The percentage of ASGR1-positive cells in LV-5TFs- or LV-HNF4A-transduced cells was examined by FACS. ***** $p < 0.05$ (vs LV-5TFs). All data are represented as means \pm SD ($n=3$).

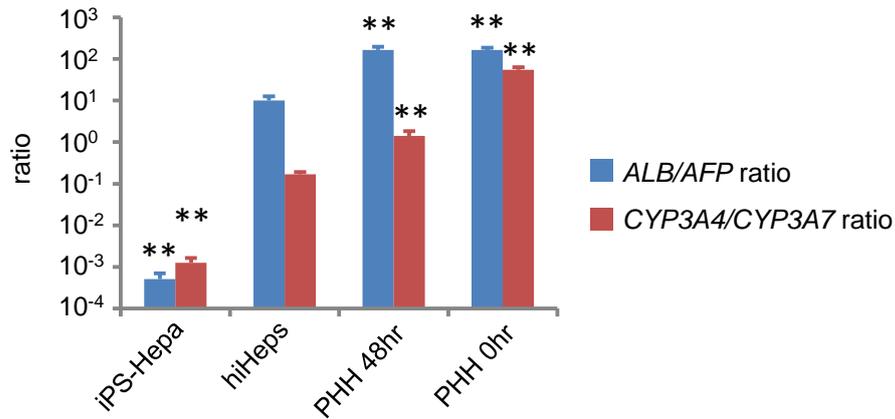


Figure S3 The expression ratios of *ALB/AFP* and *CYP3A4/CYP3A7*

MRC5 cells were transduced with LV-5TFs for 12 hr, and cultured until day 28. The hepatic gene (*ALB* and *CYP3A4*) and fetal-specific hepatic gene (*AFP* and *CYP3A7*) expression levels in iPS-Hepa, hiHeps, PHH 48hr, and PHH 0hr were then measured by real-time RT-PCR. The *ALB/AFP* and *CYP3A4/CYP3A7* ratios were calculated. ** $p < 0.01$ (vs hiHeps).

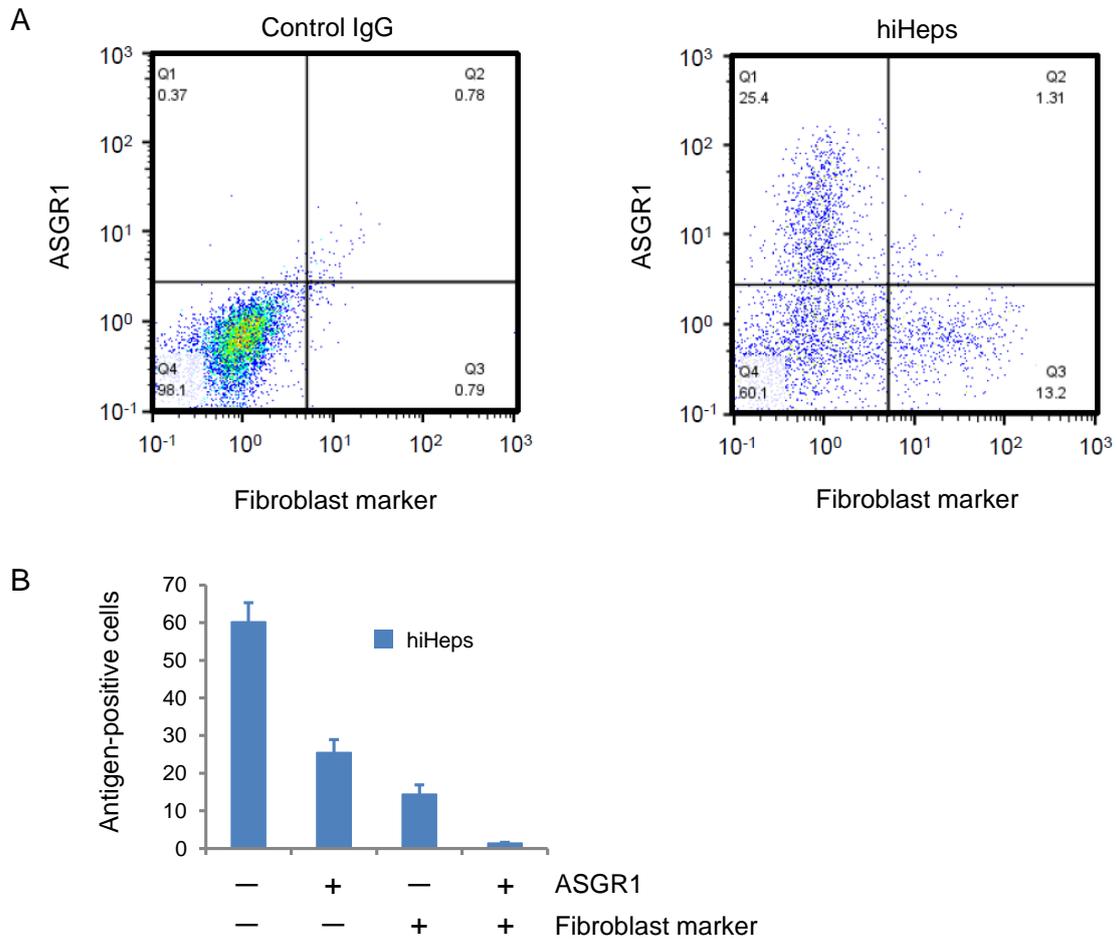


Figure S4 Immunostaining analysis of fibroblast and hepatic markers

MRC5 cells were transduced with LV-5TFs or LV-control for 12 hr, and cultured until day 28. **(A, B)** The percentages of fibroblast marker (THY-1)- and ASGR1-positive cells were examined by FACS analysis. Representative 2D plot data are shown in **figure S4A**. Quantitative data of FACS analysis are shown in **figure S4B**. All data are represented as means \pm SD ($n=3$).

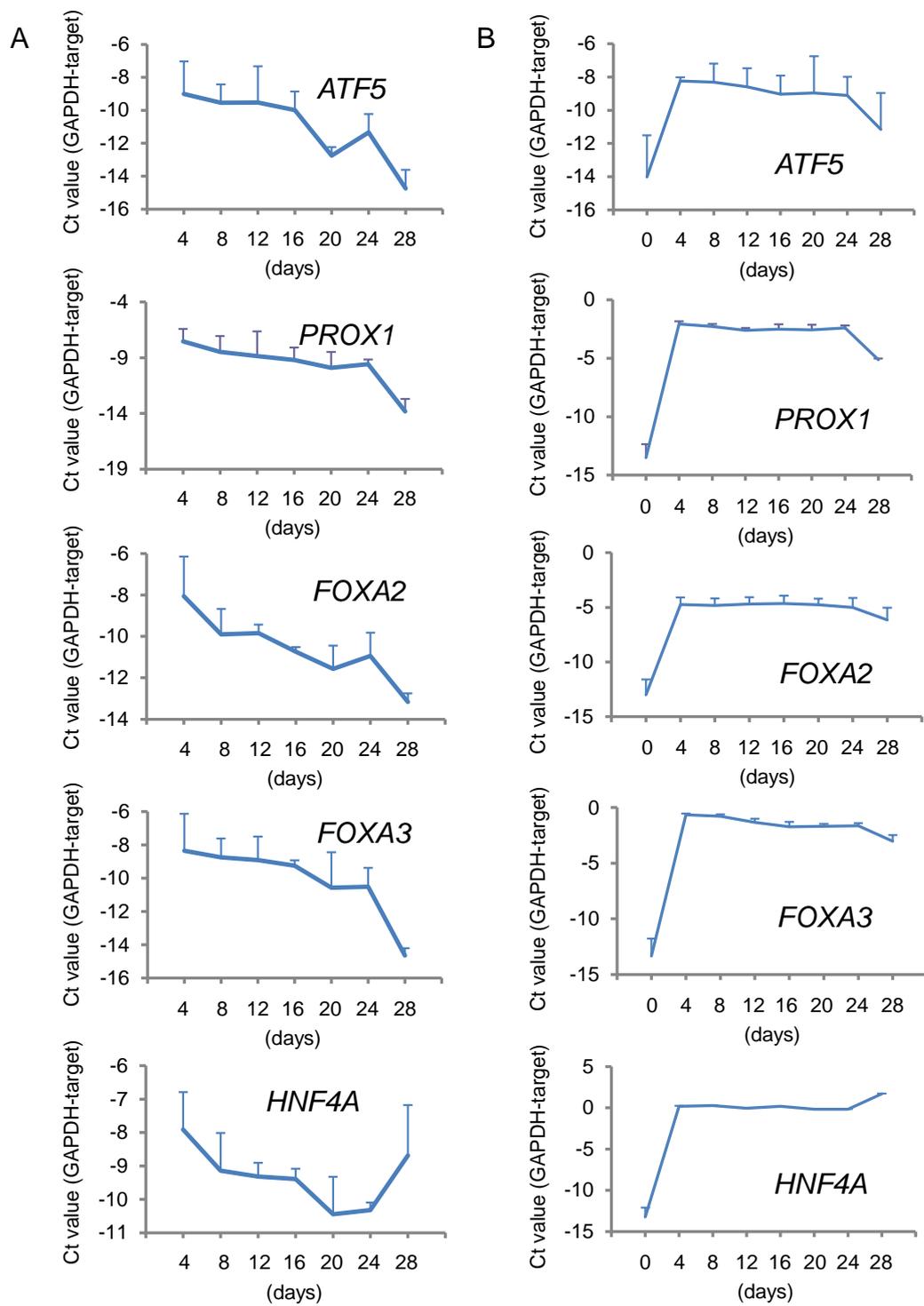


Figure S5 Exogenous and total *ATF5*, *PROX1*, *FOXA2*, *FOXA3*, and *HNF4A* expression profiles during the hepatic reprogramming

MRC5 cells were transduced with LV-5TFs for 12 hr, and cultured until day 28. (A) The exogenous gene (*ATF5*, *PROX1*, *FOXA2*, *FOXA3*, and *HNF4A*) expression levels were

measured by qRT-PCR. The gene expression levels in LV-5TF-transduced cells (day 4) were taken as 1.0. **(B)** The total (endogenous and exogenous) gene (*ATF5*, *PROX1*, *FOXA2*, *FOXA3*, and *HNF4A*) expression levels were measured by qRT-PCR. The gene expression levels in LV-5TF-transduced cells (day 0) were taken as 1.0. All data are represented as means \pm SD ($n=3$).

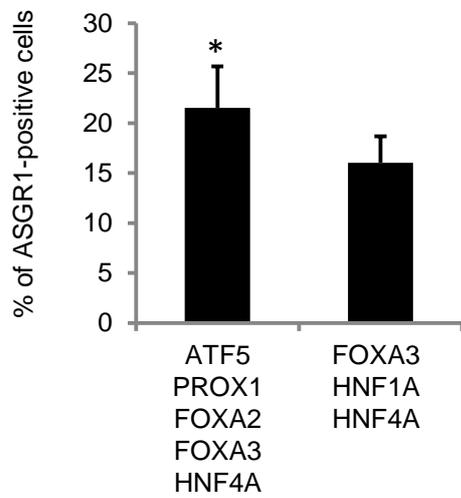


Figure S6 Comparison of hepatic direct reprogramming protocols

The hiHeps were generated by transducing MRC-5 cells with LV-5TFs (ATF5, PROX1, FOXA2, FOXA3, and HNF4A). The hiHeps were also generated by transducing MRC-5 cells with LV-3TFs (FOXA3, HNF1A, and HNF4A). The percentage of ASGR1-positive cells in hiHeps was examined by FACS. * $p < 0.05$ (vs LV-3TFs). All data are represented as means \pm SD ($n=3$).

Table S1 Primers for cloning

Gene Symbol	Genebank Number	Primers (forward/reverse; 5' to 3')
<i>ATF5</i>	NM_001193646	TGCTCGAGCCACCATGTCACTCCTGGCGACCCTG
		AAGCGGCCCGCCTAGCAGCTACGGGTCCTCTG
<i>PROX1</i>	NM_001270616	AACGAATTCGTGATGCCTGACCATGACAGCA
		TGTGCGGCCGCCTACTCATGAAGCAGCTCTTGTAGGCA
<i>CEBPA</i>	NM_004364	TCTCTCGAGCCCATGGAGTCGGCCGACTT
		AGCGGATCCTCACGCGCAGTTGCCCAT
<i>FOXA2</i>	NM_153675	GCGGAATTCAGTATGCTGGGAGCGGTGAA
		GTCGGATCCTTAAGAGGAGTTCATAATGGGCCGGGA
<i>FOXA3</i>	NM_004497	TAACTCGAGGGGATGCTGGGCTCAGTGAA
		CCCGCGGCCGCCTAGGATGCATTAAGCAAAGAGCGGG
<i>HNF1A</i>	NM_000545	GGCGAATTCGCCATGGTTTCTAAACTGAGCCAG
		AGGGCGGCCGCTTACTGGGAGGAAGAGGCCATCT
<i>HNF4A</i>	NM_178849	GAGGAATTCAGAATGCGACTCTCCAAAACCCTC
		CCAGCGGCCGCCTAGATAACTTCCTGCTTGGTGATGGT
<i>HNF6</i>	NM_004498	GCCCTCGAGACGATGAACGCGCAGCTGACCAT
		GTTGCGGCCGCTCATGCTTTGGTACAAGTGCTTGATGAAGAAG
<i>GATA4</i>	NM_002052	CTCCTCGAGACCATGTATCAGAGCTTGGCCATGG
		GAGGAATTCCTACGCAGTGATTATGTCCCCGTGAC

Table S2 Primers for real-time RT-PCR analysis

Gene Symbol	Primers (forward/reverse; 5' to 3')
<i>ALB</i>	GCACAGAATCCTTGGTGAACAG/ATGGAAGGTGAATGTTTTTCAGCA
<i>AAT</i>	ACTGTCAACTTCGGGGACAC/CATGCCTAAACGCTTCATCA
<i>CYP3A4</i>	AAGTCGCCTCGAAGATACACA/AAGGAGAGAACACTGCTCGTG
<i>COL1A1</i>	GAGGGCCAAGACGAAGACATC/CAGATCACGTCATCGCACAAC
<i>THY-1</i>	CTAGTGGACCAGAGCCTTCG/TGGAGTGCACACGTGTAGGT
<i>NANOG</i>	AGAAGGCCTCAGCACCTAC/GGCCTGATTGTTCCAGGATT
<i>Oct3/4</i>	CTTGAATCCCGAATGGAAAGGG/GTGTATATCCCAGGGTGATCCTC
<i>SOX2</i>	GGCAGCTACAGCATGATGATGCAGGAGC/CTGGTCATGGAGTTGTACTGCAGG
<i>AFP</i>	TGGGACCCGAACCTTTCCA/GGCCACATCCAGGACTAGTTTC
<i>CYP3A7</i>	AAGGTCGCCTCAAAGAGACA/TGCACTTTCTGCTGGACATC
<i>ATF5</i>	GACCGCAAGCAAAAGAAGAG/GGCCTTGTAACCTCGATGA
<i>PROX1</i>	TTGACATTGGAGTGAAAAGGACG/TGCTCAGAACCTTGGGGATTC
<i>CEBPA</i>	CCAAGAAGTCGGTGGACAAG/AGGCGGTCATTGTCACTGGT
<i>FOXA2</i>	GCGACCCCAAGACCTACAG/GGTTCTGCCGGTAGAAGGG
<i>FOXA3</i>	TCATGTAGGAGTTGAGGGGG/GAAGATGGAGGCCCATGAC
<i>HNF1A</i>	TACACCACTCTGGCAGCCACACT/CGGTGGGTACATTGGTGACAGAAC
<i>HNF4A</i>	CGTCATCGTTGCCAACACAAT/GGGCCACTCACACATCTGTC
<i>HNF6</i>	AACCCTGGAGCAAACCTCAA/AAAAAGCCCAGGTTGGTCTT
<i>GATA4</i>	CATCAAGACGGAGCCTGGCC/TGACTGTCCGGCCAAGACCAG
<i>TTR</i>	TCATCGTCTGCTCCTCCTCT/AGGTGTCATCAGCAGCCTTT
<i>CYP1A2</i>	CAATCAGGTGGTGGTGTGTCAG/GCTCCTGGACTGTTTTCTGC
<i>CYP2C9</i>	GGACAGAGACGACAAGCACA/CATCTGTGTAGGGCATGTGG
<i>CYP2C19</i>	ACTTGGAGCTGGGACAGAGA/CATCTGTGTAGGGCATGTGG
<i>CYP2D6</i>	CTTTCGCCCAACGGTCTC/TTTTGGAAGCGTAGGACCTTG
<i>CYP2E1</i>	ACCCGAGACACCATTTTCAG/TCCAGCACACACTCGTTTTTC
<i>NTCP</i>	ATCTTGGTCTGTGGCTGCTC/AGAAGGTGGAGCAGGTGGT
<i>UGT1A1</i>	TAAGTGGCTACCCCAAACG/GCTTTGCATTGTCCATCTGA
<i>GAPDH</i>	GGTGGTCTCCTCTGACTTCAACA/GTGGTCGTTGAGGGCAATG

Table S3 Antibodies for immunohistochemistry

Antigen	Type	Company
ALB	goat	Bethyl Laboratories
AAT	rabbit	Dako
Alexa Fluor 488 anti-goat IgG	donkey	Thermo Fisher Scientific
Alexa Fluor 594 anti-rabbit IgG	donkey	Thermo Fisher Scientific