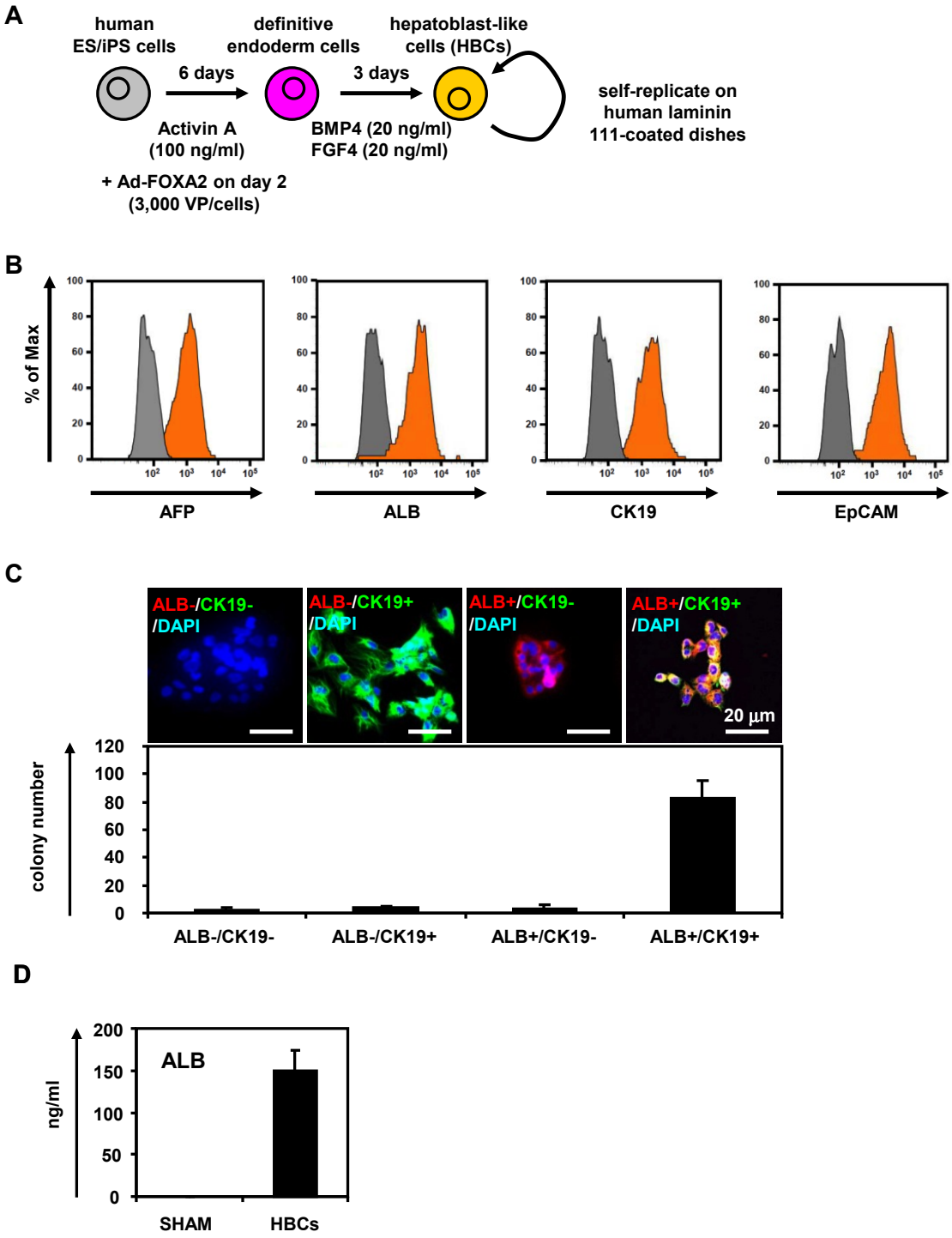

Fig. S1 The hepatoblast-like cells (HBCs) generated from hESCs were characterized.

(**A**) hESCs were differentiated into the HBCs via definitive endoderm cells. The HBCs were maintained on human LN111. (**B**) The expression levels of hepatoblast markers (AFP, ALB, CK19, and EpCAM) in the HBCs were examined by FACS

analysis. (**C**) Clonal assay of the HBC was performed. The HBCs were plated at a density of 200 cells/cm^2 on human LN111-coated 96-well plates. The colonies were separated into four groups based on the expression of ALB and CK19 (ALB and CK19 double-negative, ALB negative and CK19 positive, ALB positive and CK19 negative, and ALB and CK19 double-positive groups). The numbers represent wells in which the colony was observed in three 96-well plates (total 288 wells). Five days after plating, the cells were fixed with 4% PFA and used for double immunostaining. Nuclei were counterstained with DAPI (blue). (**D**) The HBCs were transplanted into CCl_4 (2 mL/kg)-treated Rag2/IL2 receptor gamma double-knockout mice. The human ALB level in recipient mouse serum was measured at 2 weeks after transplantation.

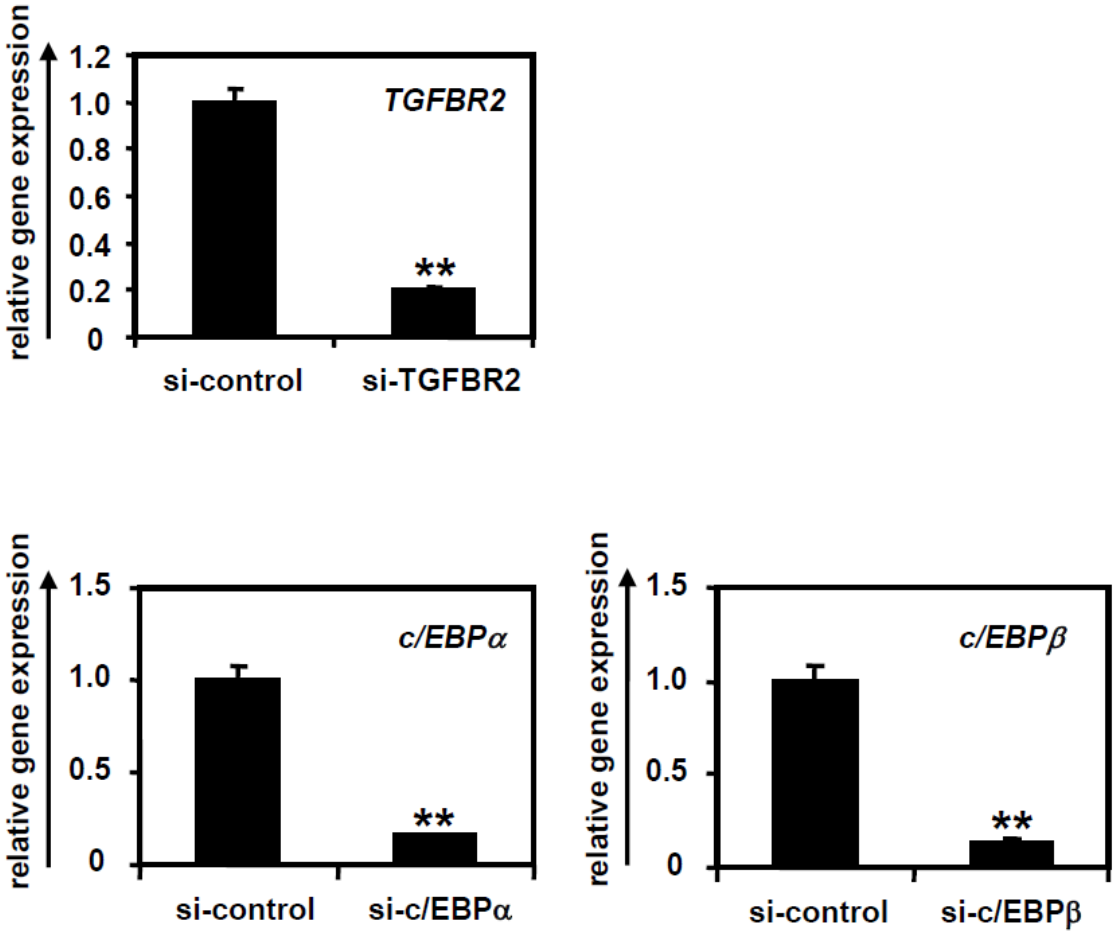

Fig. S2 c/EBPα, c/EBPβ, or TGFBR2 were knocked-down in the HBCs by si-c/EBPα, si-c/EBPβ, or si-TGFBR2 transfection, respectively.

The HBCs were transfected with 50 nM of si-control, si-c/EBPα, si-c/EBPβ, or si-TGFBR2. Two days after transfection, the gene expression levels of *c/EBPα*, *c/EBPβ*, or *TGFBR2* were examined by real-time RT-PCR in si-c/EBPα-, si-c/EBPβ-, or si-TGFBR2-transfected cells, respectively. On the y axis, the gene expression levels of *c/EBPα*, *c/EBPβ*, or *TGFBR2* in si-control-transfected cells were taken as 1.0. $^{**}P<0.01$ (compared with the si-control-transfected cells).

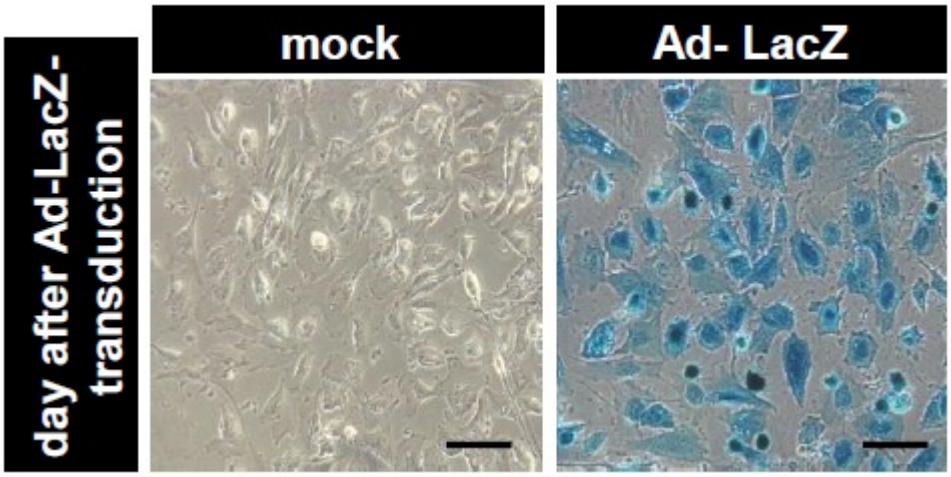

Fig. S3 Ad vectors efficiently transduced the HBCs.

The HBCs were transduced with 3,000 VP/cell of Ad-LacZ for 1.5 hr. The day after transduction, X-gal staining was performed. The scale bars represent 50 μm.

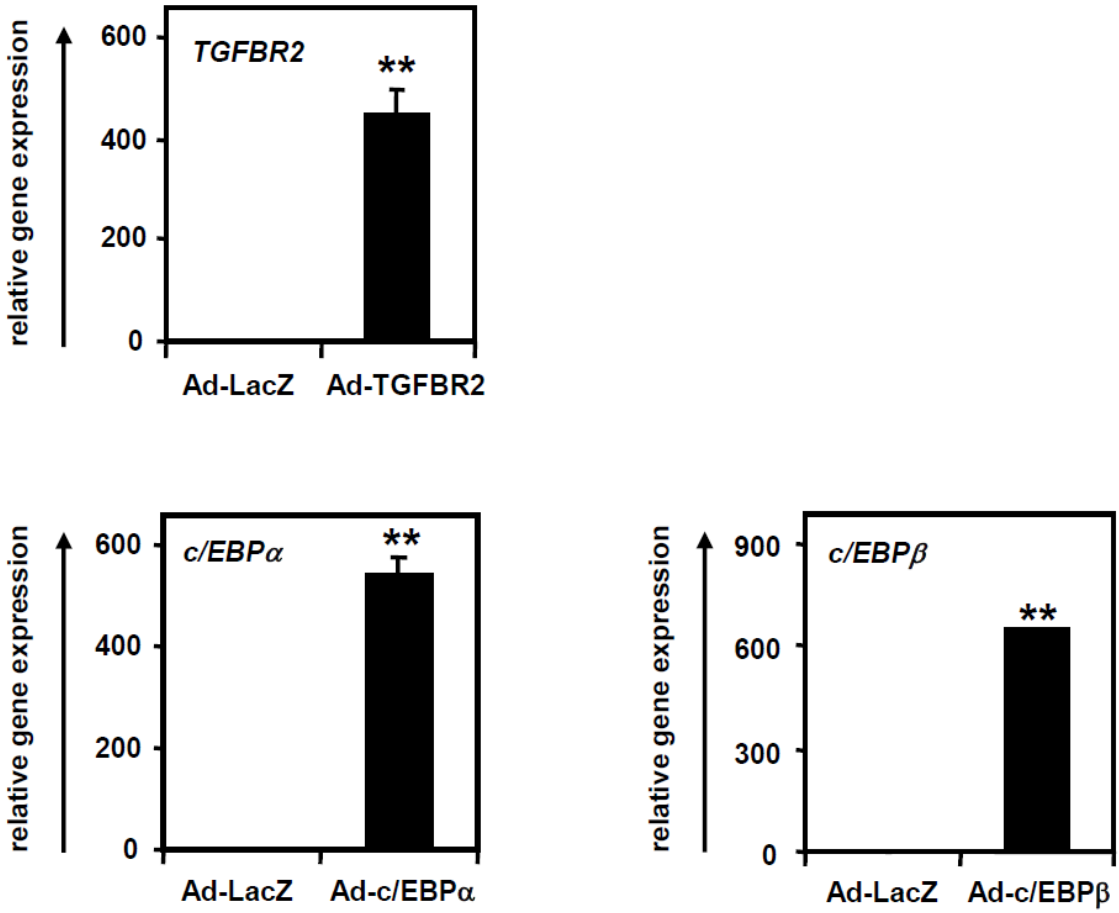

Fig. S4 c/EBPα, c/EBPβ, or TGFBR2 were overexpressed in the HBCs by Ad-c/EBPα, Ad-c/EBPβ, or Ad-TGFBR2 transduction, respectively.

The HBCs were transduced with 3,000 VP/cells of Ad-c/EBPα, Ad-c/EBPβ, or Ad-TGFBR2 for 1.5 hr. Two days after Ad vectors transduction, the gene expression levels of *c/EBPα*, *c/EBPβ*, or *TGFBR2* were examined by real-time RT-PCR in Ad-c/EBPα-, Ad-c/EBPβ-, or Ad-TGFBR2-transduced cells, respectively. On the y axis, the gene expression levels of *c/EBPα*, *c/EBPβ*, or *TGFBR2* in Ad-LacZ-transduced cells were taken as 1.0. $^{**}P<0.01$ (compared with the Ad-LacZ-transfected cells).

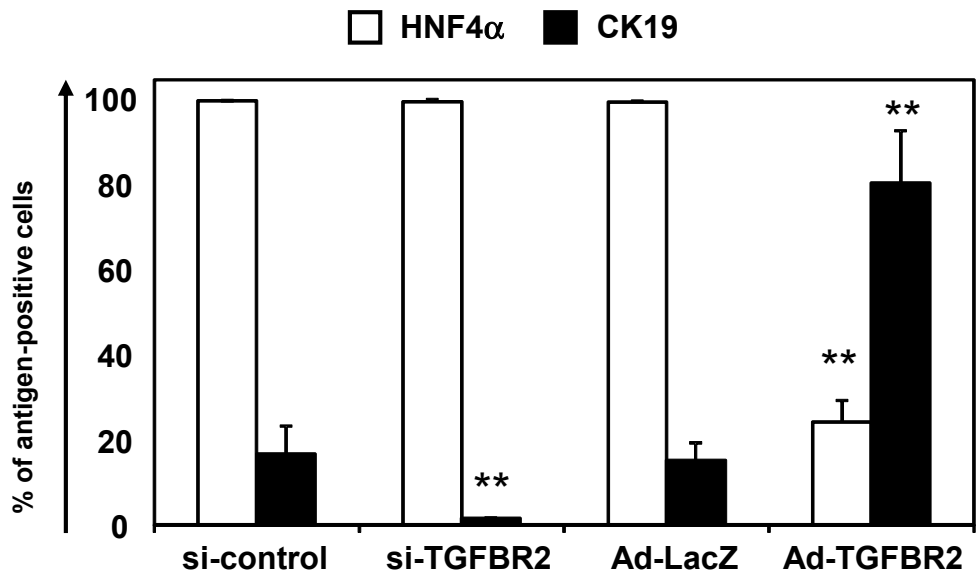

Fig. S5 TGFBR2 overexpression or knockdown in the HBCs promotes cholangiocyte or hepatocyte differentiation, respectively.

The si-control-, si-TGFBR2-, Ad-LacZ- or Ad-TGFBR2-transduced HBCs (total of 1.0×10^6 cells) were transplanted into CCl_4 (2 mL/kg)-treated Rag2/IL2 receptor gamma double knockout mice by intrasplenical injection. Expressions of HNF4α and CK19 were examined by immunohistochemistry at 2 weeks after transplantation. Semiquantitative analysis of the immunofluorescent staining was performed in the human cell clusters. **P*<0.05; ***P*<0.01.

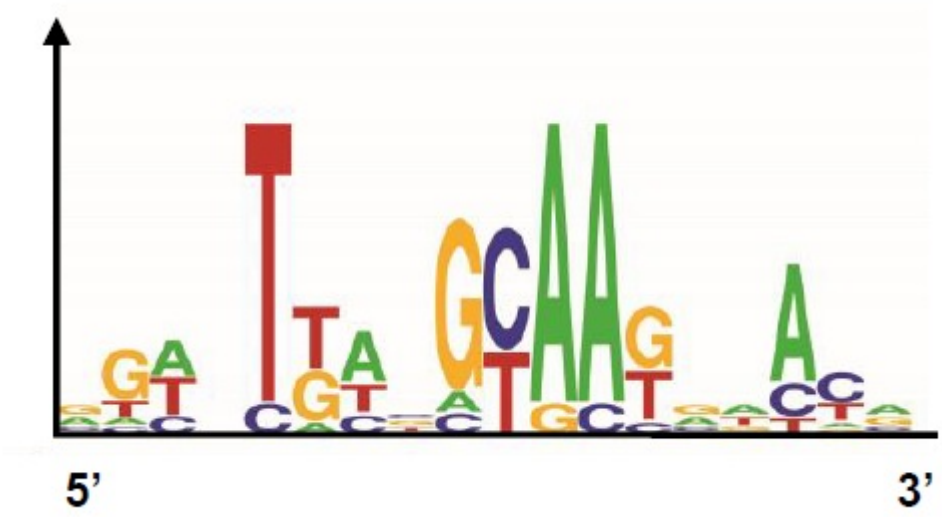

Fig. S6 c/EBP-binding site on the TGFBR2 promoter region

The consensus sequence of the c/EBP-binding site is described. (http://www.cbil.upenn.edu/cgi-bin/tess/tess).

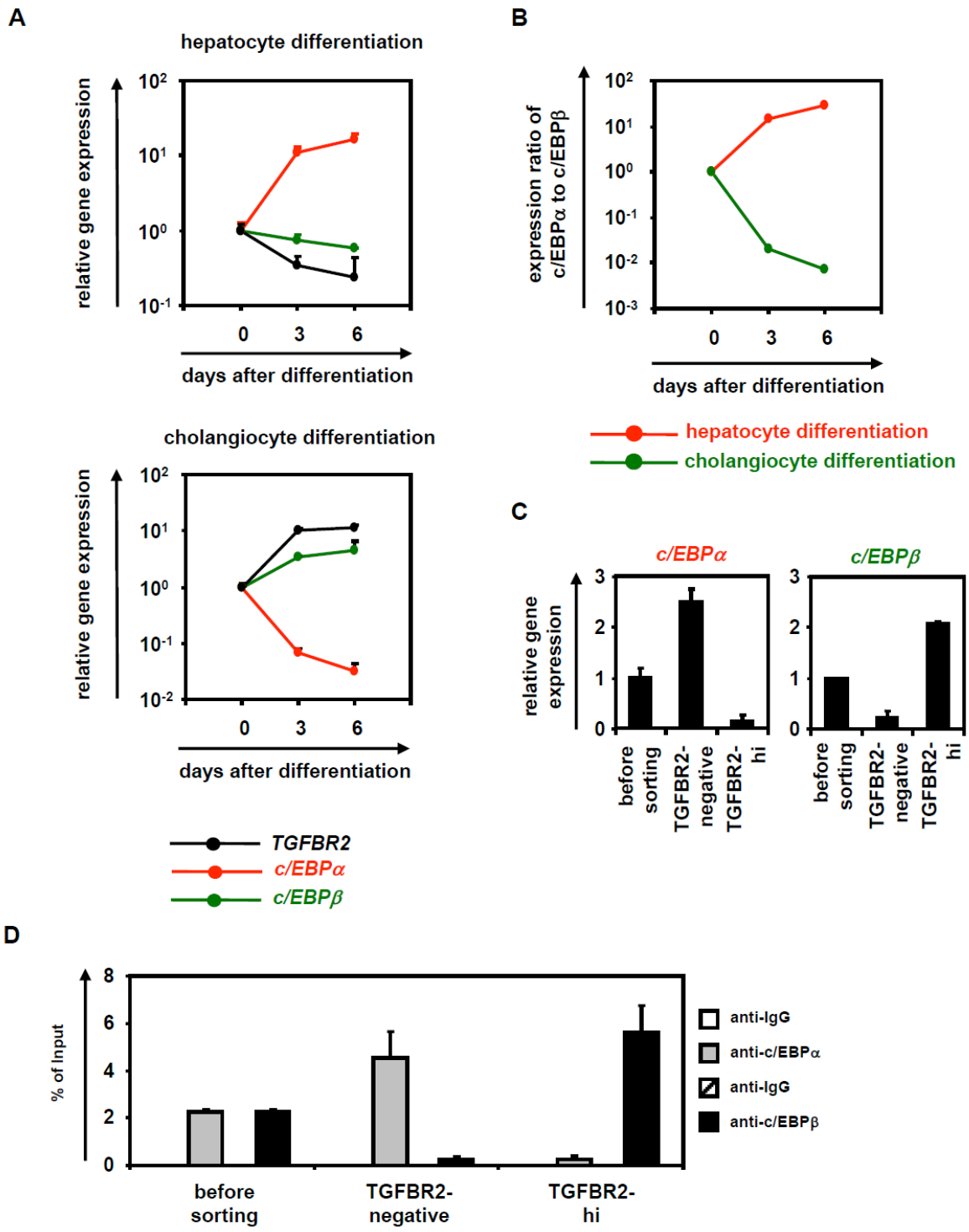

Fig. S7 Temporal gene expression levels of *TGFBR2*, *c/EBPα*, and *c/EBPβ* in hepatocyte and cholangiocyte differentiation.

The HBCs were differentiated into hepatocyte-like cells or cholangiocyte-like cells as shown in figure 1A. (**A**) Temporal gene expression levels of *TGFBR2*,

c/EBPα, and *c/EBPβ* in hepatocyte differentiation and cholangiocyte differentiation of the HBCs were examined by real-time RT-PCR. On the y axis, the gene expression levels in the HBCs were taken as 1.0. (**B**) The temporal ratio of c/EBPα to c/EBPβ was demonstrated in hepatocyte and cholangiocyte differentiation. The ratio of c/EBPα to c/EBPβ in the HBCs was taken as 1.0. (C) The HBCs were cultured on Matrigel for 5 days, and then the expression level of TGFBR2 was examined by FACS analysis. TGFBR2-negative, -lo, and –hi populations were collected as described in figure 1F. Real-time RT-PCR analysis was performed in three populations (before sorting, TGFBR-negative, and TGFBR2-hi) to measure the expression levels of *c/EBPα* and *c/EBPβ*. (**D**) The recruitment of c/EBPα or c/EBPβ to the TGFBR2 promoter region in three populations (before sorting, TGFBR-negative, and TGFBR2-hi) was examined by ChIP assay.

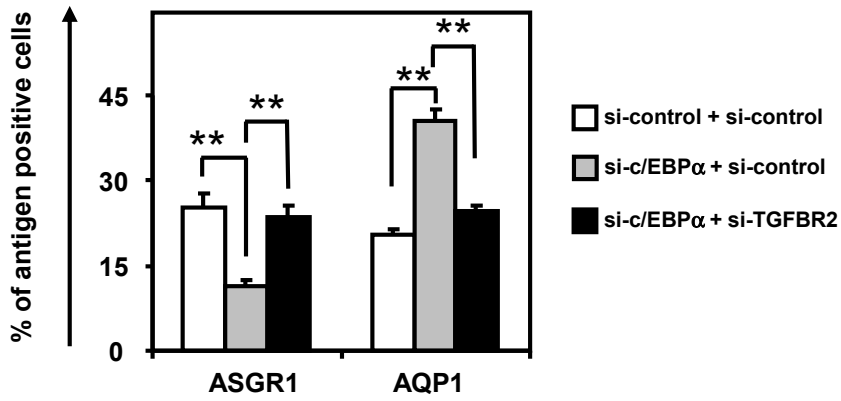

Fig. S8 Inhibition of hepatocyte differentiation by si-c/EBPα transfection was rescued by si-TGFBR2 transfection.

The HBCs were transfected with 50 nM of each of si-control + si-control, si-c/EBPα + si-control, or si-c/EBPα + si-TGFBR2 and cultured with the differentiation hESF-DIF medium for 10 days. The efficiency of hepatocyte or cholangiocyte differentiation was measured by estimating the percentage of ASGR1- or AQP1-positive cells, respectively, using FACS analysis. **P*<0.05; ***P*<0.01.

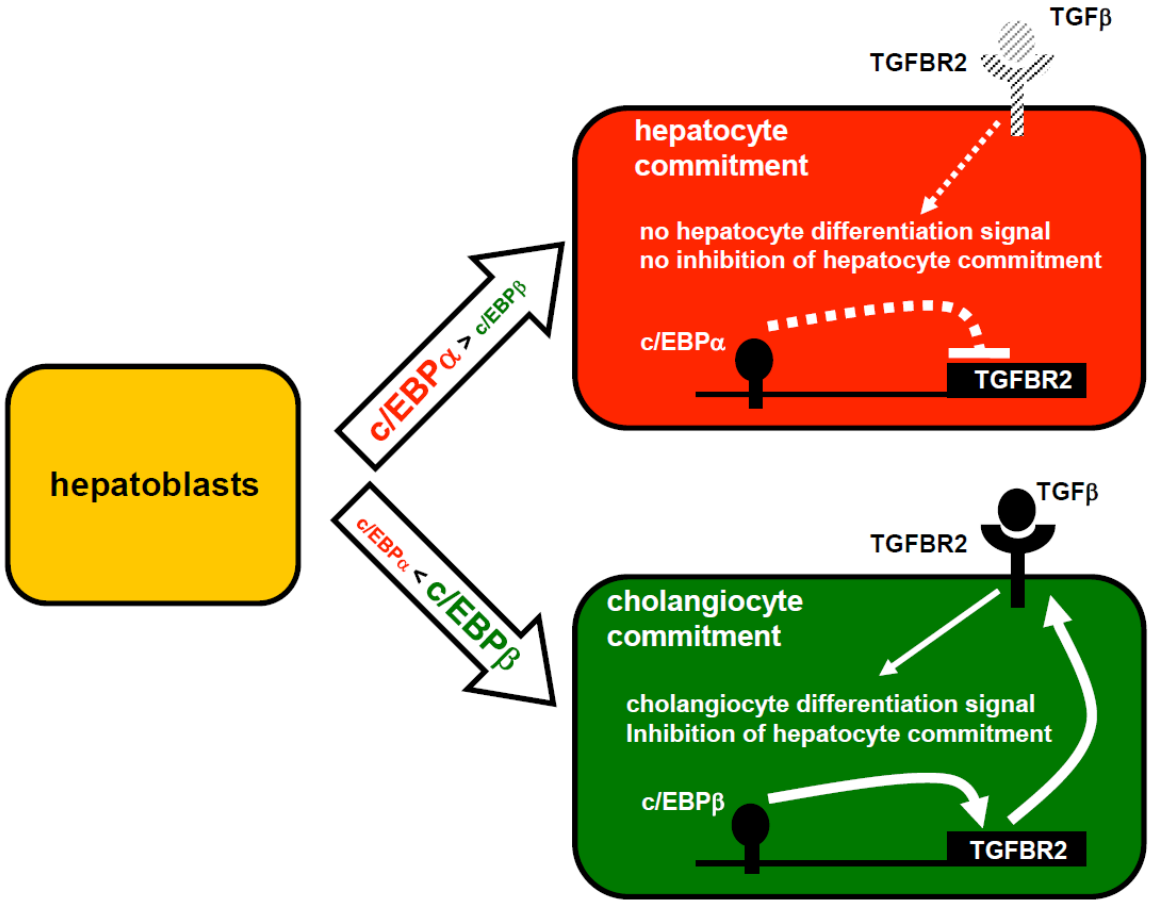

Fig. S9 The lineage segregation of hepatoblasts might be explained by c/EBP-mediated control of TGFBR2 expression.

In hepatocyte differentiation from hepatoblasts, c/EBPα promotes hepatocyte differentiation via negative regulation of TGFBR2 expression. On the other hand, c/EBPβ promotes cholangiocyte differentiation via positive regulation of TGFBR2 expression in cholangiocyte differentiation.

Supplemental Table 1 The primary antibodies used in this study

Antigen	Species	Company (catalog number)	Dilution
CK19	rabbit	Abcam (ab52625)	1/250
AFP	mouse	Cell Signaling (#3903)	1/100
c/EBPβ	rabbit	Santa Cruz Biotechnology (sc-150AC)	1/50
ALB (ELISA)	goat	Bethyl Laboratories (E80-129)	
ALB (FCM)	rabbit	Abcam (ab135575)	1/40
ALB (IHC)	goat	Santa Cruz Biotechnology (sc-46293)	1/200
c/EBPα	rabbit	Abcam (ab40764)	1/50
HNF4α	abcm	Abcam (ab36175)	1/100
TGFBR2	mouse	Santa Cruz Biotechnology (sc-17799)	1/50
ASGR1	goat	Santa Cruz Biotechnology (sc-13467)	1/50
CYP3A4	goat	Santa Cruz Biotechnology (sc-27639)	1/200
AQP1	mouse	Abcam (ab9566)	1/40
EpCAM	mouse	Militenyi Biotec (130-091-254)	1/50

Supplemental Table 2 The secondary antibodies used in this study

Antigen	label	Company	Species	Dilution
rabbit IgG	alexa fluor 488	Molecular Probes	goat	1/1000
rabbit IgG	alexa fluor 488	Molecular Probes	chicken	1/1000
mouse IgG	alexa fluor 488	Molecular Probes	rabbit	1/1000
goat IgG	alexa fluor 488	Molecular Probes	rabbit	1/1000
rabbit IgG	alexa fluor 594	Molecular Probes	mouse	1/1000
goat IgG	alexa fluor 594	Molecular Probes	mouse	1/1000
goat IgG	alexa fluor 594	Molecular Probes	chicken	1/1000
goat IgG	alexa fluor 594	Molecular Probes	donkey	1/1000
mouse IgG	alexa fluor 594	Molecular Probes	chicken	1/1000

Supplemental Table 3 The primers used for real-time RT-PCR in this study

Genes	Primers (forward/reverse; 5' to 3')
CK7	AGACGGAGTTGACAGAGCTG/GGATGGCCCGGTTCATCTC
CK19	CTCCCGCGACTACAGCCACT/TCAGCTCATCCAGCACCCTG
HES1	ATGGAGAAAAATTCCTCGTCCC/TTCAGAGCATCCAAAATCAGTGT
SOX9	TTTCCAAGACACAAACATGA/AAAGTCCAGTTTCTCGTTGA
integrin β4	GCAGCTTCCAAATCACAGAGG/CCAGATCATCGGACATGGAGTT
TO	GGCAGCGAAGAAGACAAATC/TCGAACAGAATCCAACTCCC
αAT	ACTGTCAACTTCGGGGACAC/CATGCCTAAACGCTTCATCA
ALB	GCACAGAATCCTTGGTGAACAG/ATGGAAGGTGAATGTTTTCAGCA
TGFBR2	GGAAACTTGACTGCACCGTT/CTGCACATCGTCCTGTGG
c/EBPα	TTCACATTGCACAAGGCACT/GAGGGACCGGAGTTATGACA
c/EBPβ	CGTGTACACACGCGTTCAG/CTCTCTGCTTCTCCCTCTGC
HNF6	CAAACCCTGGAGCAAACTCAA/TGTGTTGCCTCTATCCTTCCC
HNF1β	ACCAAGCCGGTCTTCCATACT/GGTGTGTCATAGTCGTCGCC
CYP2D6	CTTTCGCCCCAACGGTCTC/TTTTGGAAGCGTAGGACCTTG
TTR	TCATCGTCTGCTCCTCCTCT/AGGTGTCATCAGCAGCCTTT
HNF1α	AACACCTCAACAAGGGCACTC/CCCCACTTGAAACGGTTCCT
CYP3A4	AAGTCGCCTCGAAGATACACA/AAGGAGAGAACACTGCTCGTG
mouse αAT	TTGCTCGACACAACATGGAAT/ACGTCCCAGTTTGACATCTCT
mouse CYP7A1	GCTGTGGTAGTGAGCTGTTG/GTTGTCCAAAGGAGGTTCACC
mouse AQP1	AGGCTTCAATTACCCACTGGA/CTTTGGGCCAGAGTAGCGAT
mouse integrin β4	AGAGCTGTACCGAGTGCATC/TGGTGTCGATCTGGGTGTTCT

Supplemental Table 4 The primers used for ChIP assay in this study

	Primers (forward/reverse; 5' to 3')
c/EBP binding site A	TCACAACTTTCTAAGTCCCAATTTT/ACTGAGGCAGGGACTGTGTC
c/EBP binding site B	AACTGAAATGTCTTCCTTTTTCAA/CAGGAGGAGTAGAGCCAGCA
c/EBP binding site C	GCCACATTGTGTTTTCAGGA/ TTAGCCGAGAATGATGTCACC
c/EBP binding site D	CCAGAGGGCTGTACAGAATCA/ CCAGATTTGCCCAAGACATT
c/EBP binding site E	TGCCTACTGGGTGCTAGAGG/AACCTTCAGAGACAGCGATCA
β-actin	CCGGCGGGGTCTTTGTCTGAGC/GGGCCGGCCGCGTTATTACCA