Chromatin immunoprecipitation (ChIP) assay

The ChIP assay kit was purchased from Upstate. Cells were crosslinked using formaldehyde at a final concentration of 1% at 37°C for 10 minutes, and then genomic DNA was fragmented by sonicator. The resulting DNA-protein complexes were immunoprecipitated using the antibodies described in supplementary material Table S1 or control IgG as described in supplementary material Table S2. The precipitated DNA fragments were analyzed by real-time RT-PCR using the primers shown in supplementary material Table S4 to amplify the *TGFBR2* promoter region including the c/EBP binding sites or β-actin locus as a control. The results of quantitative ChIP analysis (Fig. 5A) were expressed as the amount of amplified *TGFBR2* promoter region relative to input DNA taken as 100%.

Statistical analysis

Statistical analysis was performed using an unpaired two-tailed Student's *t*-test. All data are represented as mean ± s.d. (n=3).

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Competing interests

The authors declare no competing financial interests.

Author contributions

K. Takayama, K.K. and H.M. developed the concepts or approach; K. Takayama, Y.N., K.O., H.O. and T.Y. performed experiments; K. Takayama, K.K., M.I., K. Tashiro, F.S., T.H., T.O., M.F.K. and H.M. performed data analysis; K. Takayama, K.K. and H.M. prepared or edited the manuscript prior to submission.

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Supplementary material

Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.103168/-/DC1

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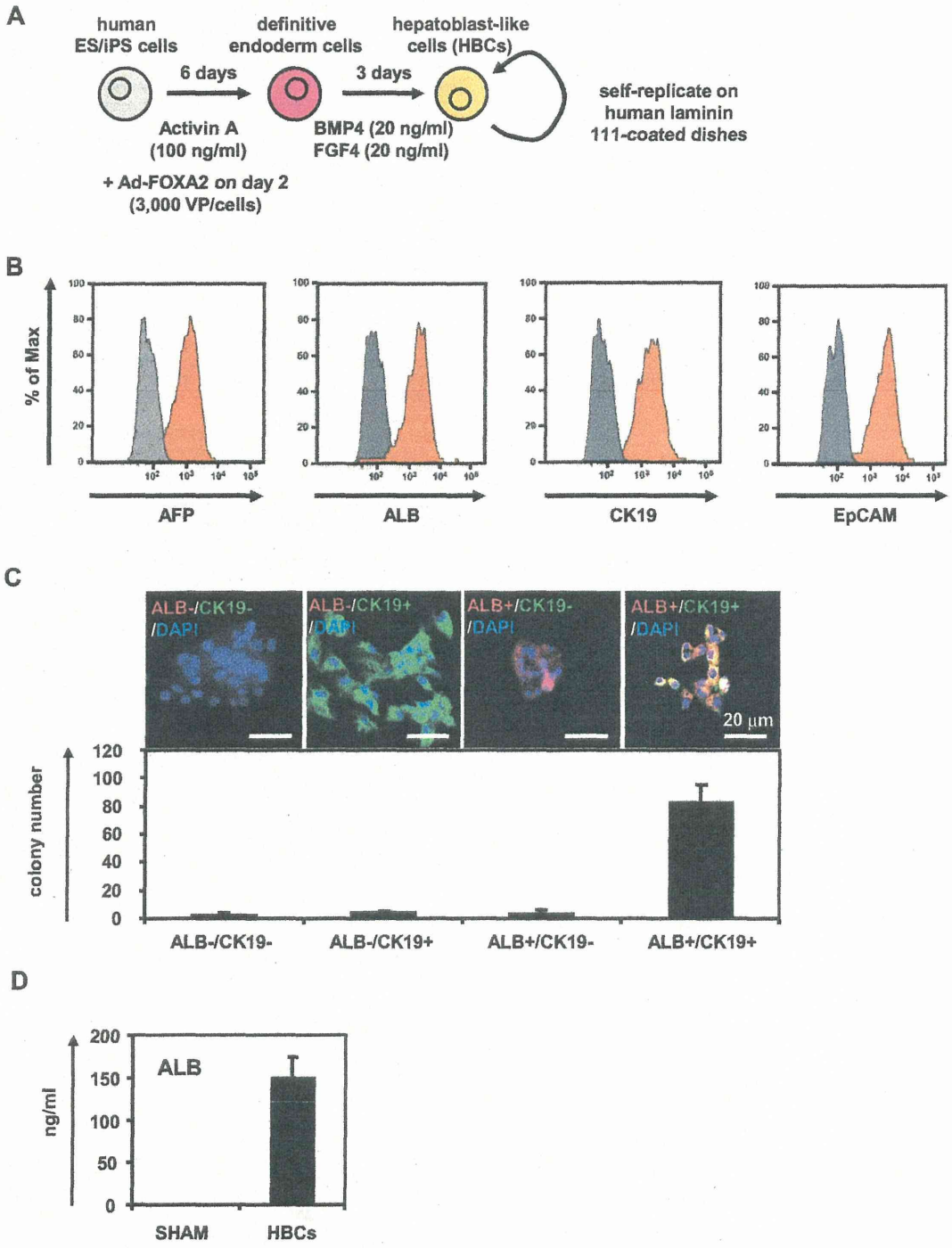

Fig. S1 The hepatoblast-like cells (HBCs) generated from hESCs were characterized.

(**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.

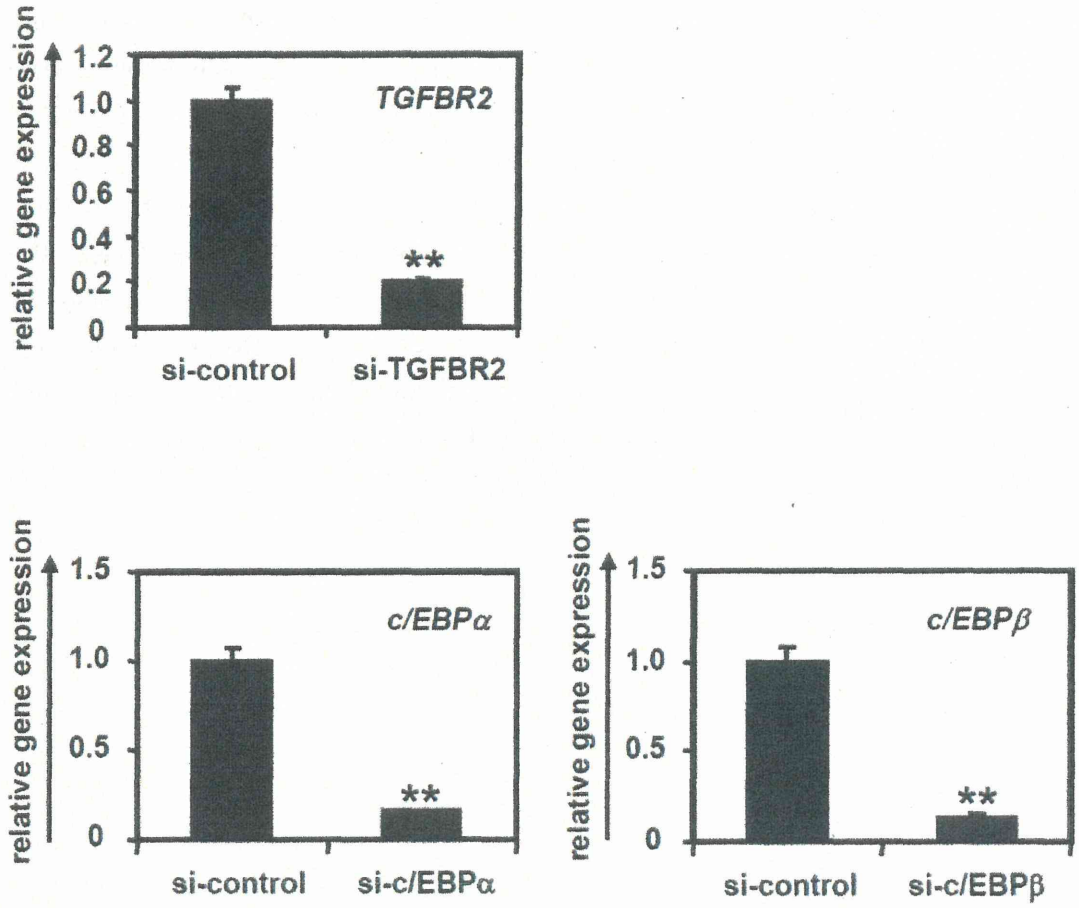

Fig. S2 c/EBPα, c/EBPβ, or TGFBR2 were knocked-down in the HBCs by si-c/EBPα, si-c/EBPβ, or si-TGFBR2 transfection, respectively.

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).

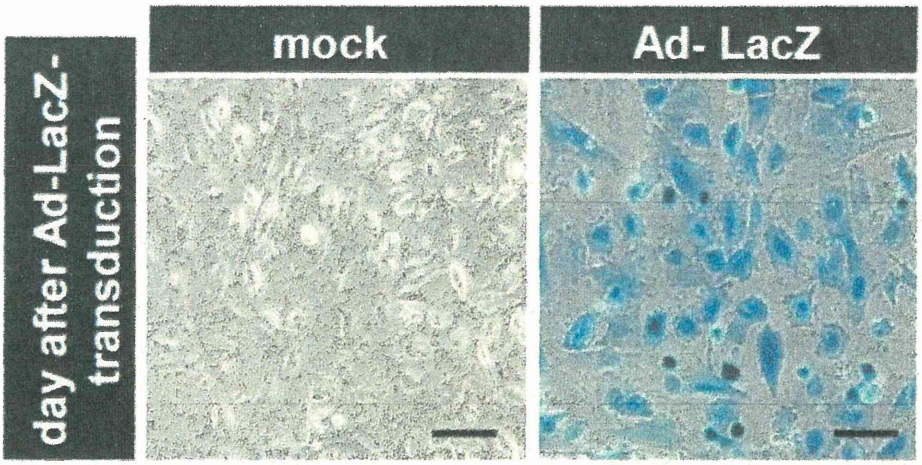

Fig. S3 Ad vectors efficiently transduced the HBCs.

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.

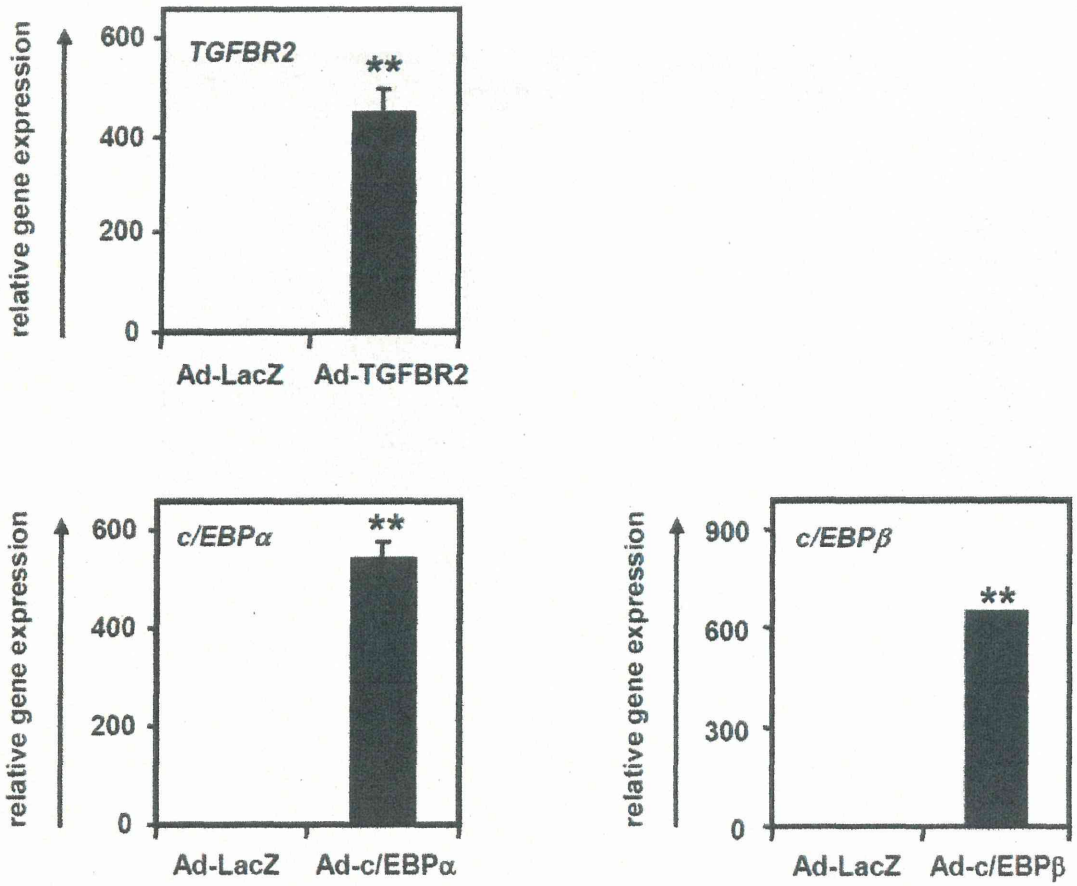

Fig. S4 c/EBPα, c/EBPβ, or TGFBR2 were overexpressed in the HBCs by Ad-c/EBPα, Ad-c/EBPβ, or Ad-TGFBR2 transduction, respectively.

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).

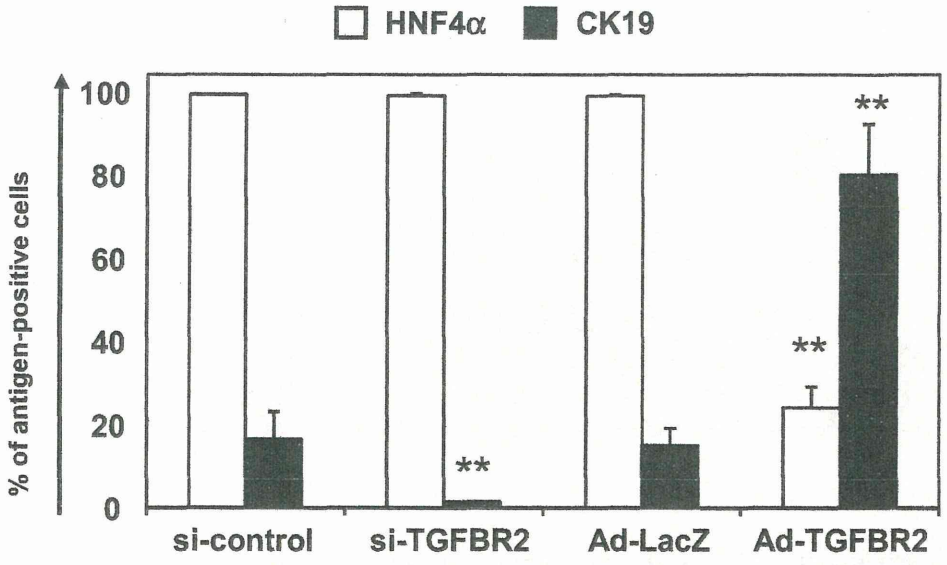

Fig. S5 TGFBR2 overexpression or knockdown in the HBCs promotes cholangiocyte or hepatocyte differentiation, respectively.

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).

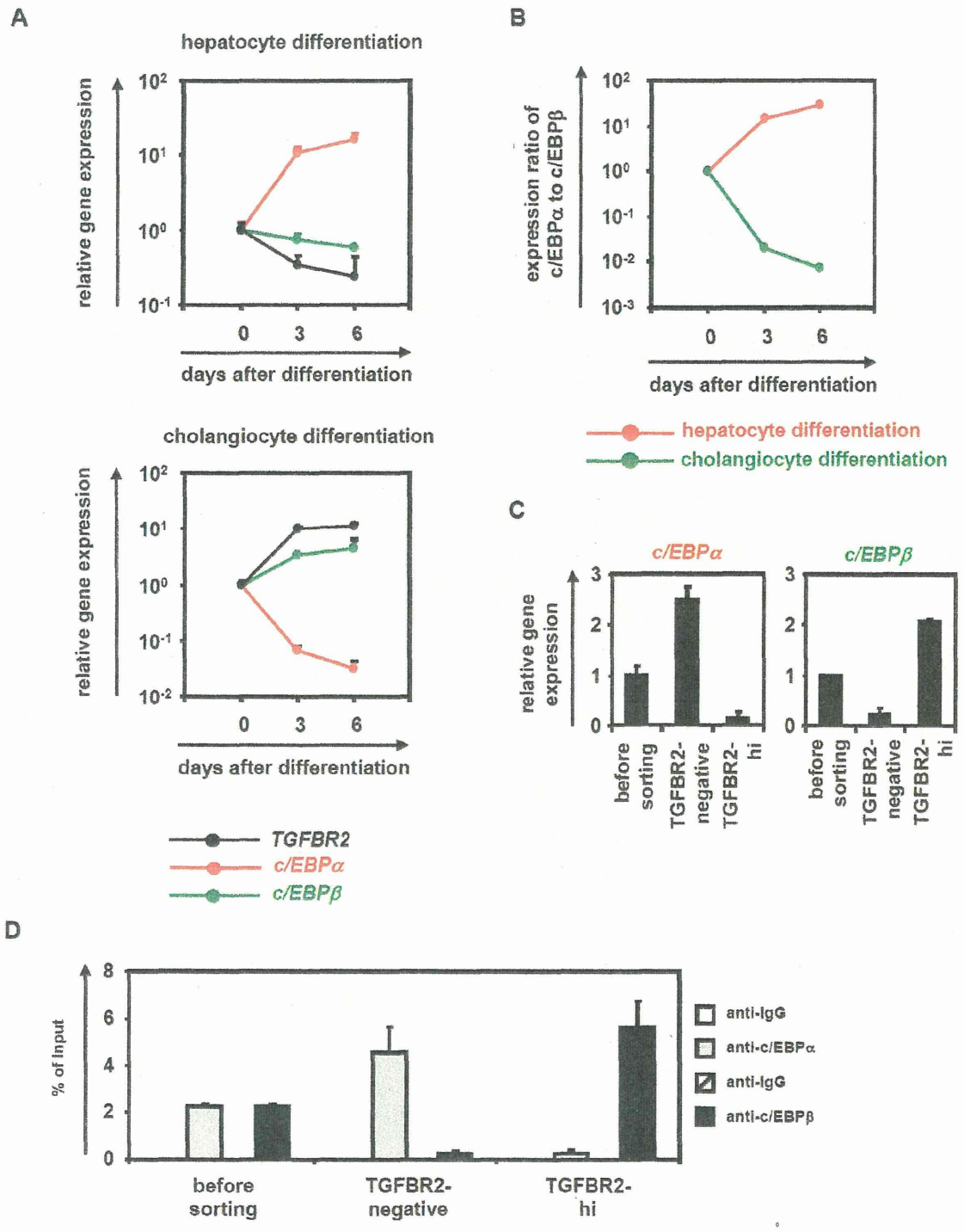

Fig. S7 Temporal gene expression levels of *TGFBR2*, *c/EBPα*, and *c/EBPβ* in hepatocyte and cholangiocyte differentiation.

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.