**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Fig. S1.** KLF4 protein expression in human HCC specimens and its association with tumor grade. TMA immunostainingwas conducted using a specific anti-KLF4 antibody. (***A***) Distinct levels of KLF4 staining in HCC specimens correlated with well/moderate (grade 1-2) and poor (grade 3) differentiation. Of note is that loss of KLF4 expression was more prominent in grade 3 than in grade 1-2 tumors. (***B***) KLF4 expression pattern in a case of HCC with heterogeneous tumor differentiation. The low-grade (grade 1) tumor area had a higher percentage of KLF4-positive nuclear and stronger cytoplasmic stained HCC cells than did the moderate-grade (grade 2) area. (***C***) Relationship between the expression levels of KLF4 and tumor grades were analyzed (n=99).

**Supplementary Fig. S2.** KLF4 protein expression in human HCC and paired nontumor tissue specimens. (***A***) Overview of KLF4 staining in the representative TMAs. T, tumor; N, paired nontumor tissue. Of note is that KLF4 staining in the tumor specimens was weaker than that in the paired nontumor tissue specimens. (***B***) Representative photos of strong KLF4 staining of nontumor liver tissue specimens from two patients.

**Supplementary Fig. S3.** Expression of differentiation-associated markers in microdissected well-differentiated (grade 1) and poorly differentiated (grade 3) HCC cells in frozen tissue sesctions. (***A***) Well-differentiated and poorly differentiated HCC cells were purified via LCM. (***B***) RT-PCR analysis of total RNA extracted from the microdissected grade 1 and 3 tumor cells to examine the patterns of expression of the indicated differentiation-associated markers.

**Supplementary Fig. S4.** Characterization of ZFD-deleted KLF4 protein (KLF4ΔZFD). (***A***) Schematic structure of KLF4 and KLF4ΔZFD protein (left panel). AD, activation domain; ID, inhibitory domain; NLS, nuclear localization signal sequence. SNU387 cells were transfected with a control pcDNA3.1, FLAG-tagged KLF4, or FLAG-tagged KLF4ΔZFD-expressing vector. Total protein lysates were harvested and subjected to Western blot analysis with an anti-FLAG antibody (right panel). (***B***) PANC-1 cells were transfected with EGFP-tagged KLF4-expressing and EGFP-tagged KLF4ΔZFD-expressing vectors. Protein localization was observed under a [fluorescence](app:ds:fluorescence) [microscope](app:ds:microscope) 48 hours after transfection. (***C***) PANC-1 cells were co-transfected with the p27Kip1 promoter reporter p27-N-MB435 and the indicated KLF4/KLF4ΔZFD-expressing plasmids or empty control vectors. The relative promoter activities were measured 48 hours after transfection.

**Supplementary Fig. S5.** The influence of treatment with sodium butyrate on the expression of LETFs and KLF4. Expression of LETFs and KLF4 in SNU387 cells treated with sodium butyrate (NaBu) at different doses as measured using RT-PCR (upper panel). The relative level of KLF4 and HNF-6 expression was quantified and normalized according to GAPDH expression (KLF4 expression in the 0 mM group and HNF-6 expression in the 1 mM group were used as references; lower panels).

**Supplementary Fig. S6.** HNF-6 and KLF4 protein expression in human HCC specimens and their association with tumor grade. TMA immunostaining was performed using a specific anti-KLF4 or anti-HNF-6 antibody. (***A***) Representative HNF-6 staining pattern in nontumor liver tissue and well-differentiated, moderately differentiated, and poorly differentiated HCC specimens. Expression of HNF-6 decreased along with HCC dedifferentiation. (***B***) HNF-6 expression in a case of HCC with heterogeneous tumor differentiation. The low-grade (grade 1) tumor area had a higher percentage of HNF-6–positive stained HCC cells than did the moderate-grade (grade 2) area. G1, grade 1; G2, grade 2; red dotted line, borderline between normal cells and tumor cells. (***C***) Relationship between the expression levels of HNF6 and tumor grades were analyzed (n=99).

**Supplementary Fig. S7.** Alb-Cre–mediated KLF4 deletion in the murine liver. Genotyping of *Alb-Cre+; Klf4fl/fl* and *Alb-Cre‒; Klf4fl/fl* mice at the age of 20 weeks using liver (L), pancreas (P), and tail (T) tissues is shown. PCR screening revealed a 370-bp fragment of the *Alb-Cre* transgene, a 296-bp band of the Klf4 floxed allele (Klf4-fl), and a 425-bp band of the Klf4 deleted allele (Klf4-Δ).

**Supplementary Fig. S8.** Basic activity of HNF-6 promoter reporters in HCC cells. (***A***) Schematic structure of HNF-6 promoter reporters. (***B***) The HNF-6 promoter reporters were transfected into SNU387 (left), SK-HEP-1 (middle), and PLC/PRF/5 (right) cells in triplicate. Transfection of these cells with pGL3-basic was used as a negative control. The relative promoter activities were measured 36 hours after transfection.

**Supplementary Fig. S9.** Endogenous binding of KLF4 to the HNF-6 promoter. (***A***) The nucleotide positions and sequences of the PCR primer pair (P1 and P2) flanking the potential KLF4 binding sites in the ChIP assay are shown. (***B***) ChIP assays were performed using chromatins extracted from SNU387, SK-HEP-1, and PLC/PRF/5 cells with a specific anti-KLF4 antibody or a control IgG. Chromatin fragments without IgG or the antibody were used as input controls. PCR was performed using two sets of primers (P1 and P2) as described in Supplementary Methods.

**Supplementary Fig. S10.** Prognostic significance of KLF4 and HNF-6 expression in HCC patients after OLT. (A) Loss of KLF4 expression was associated with reduced OS rate (left) and short RFS duration (right) after OLT (P<.001 for both [log-rank test]). The 1-, 3-, and 5-year survival rates in the groups are indicated and were calculated using the Kaplan-Meier method. (B) Loss of HNF-6 expression was associated with reduced OS rate (left) and short RFS duration (right) in patients after liver transplantation (P=.001 and .021, respectively [log-rank test]). The 1-, 3-, and 5-year survival rates in the groups are indicated and were calculated using the Kaplan-Meier method. N/W, negative/weak; M, moderate; S, strong. (C) OS curves (left) and RFS curves (right) in transplanted HCC patients with tumors within the Milan criteria stratified according to KLF4 expression status. Survival-rate differences between groups were calculated using the log-rank test. (D) OS curves (left) and RFS curves (right) in transplanted HCC patients with tumors exceeding the Milan criteria stratified according to KLF4 expression status. Survival-rate differences between groups were calculated using the log-rank test. Arrows, 5-year OS rate (left) and RFS rate (right) calculated using the Kaplan-Meier method.