**Supplemental Figure legends**

**Supplemental Fig. 1 The human HCC cell lines that expressed EGFP under the control of the human K19 promoter.** (A) RT-PCR analysis of four HCC cell lines (left panel). Phase- contrast images of the stable transfectants (right panel). The images of each cell line showed the same visual field. Scale bar represents 100 µm. (B) Immunocytochemistry assay of stable transfectants and the ratio of K19+EGFP+ cells to all K19+ cells (middle and right panels), and PCR analysis of genomic DNA of stable transfectants (left panel). In all cell lines, pEGFP1 gene was integrated into genomic DNA of each cell line. (C) FACS analyses of stable transfectants (upper panel), RT-PCR analysis of sorted EGFP+ and EGFP− cells (lower left panel), and qPCR analyses of genomic DNA of sorted EGFP+ and EGFP− cells for pEGFP1 gene (lower right panel, Student’s *t*-test, n.s.; not significant). (D) qRT-PCR analyses of control-siRNA transfected K19+ cells and K19-siRNA transfected K19+ cells (left panel, Student’s *t*-test, *P* < 0.05). Data are shown as the mean ± SD. Phase-contrast images obtained at 0 h or 24 h after the scratch and of the remained wounds at 24 h after the scratch in wound-healing assays of control-siRNA transfected K19+ cells and K19-siRNA transfected K19+ cells (right upper panel, Student’s *t*-test, *P* < 0.05). Original magnification was 20×.

**Supplemental Fig. 2 Cancer stem cell properties of K19+ PLC/PRF/5 and Hep3B cells *in vitro*.** (A) Single-cell culture analyses of K19+ and K19− cells. In both cell lines, single K19+ cells sorted from P5 quadrangle generated both K19+ and K19− cell fractions, whereas single K19− cells sorted from P4 quadrangle produced only K19− cell fractions. The vertical axis indicates 7-amino-actinomycin D fluorescence and the horizontal axis indicates the intensity of enhanced green fluorescence protein-K19. (B) Cell proliferation assays of K19+ and K19− cells (repeated-measures ANOVA, *P* < 0.01). (C) Colony numbers and light microscopic images in the anchorage-independent growth assay (left and middle panels, Student’s *t*-test, *P* < 0.05), and phase-contrast images in the sphere-forming assay (right panel). The original magnification was 20×. (D) Half-maximal inhibitory concentration (IC50) of 5-fluorouracil (5-FU) values (left panel, *F*-test, *P* < 0.01) and qRT-PCR analyses of K19+ and K19− cells for multidrug resistance protein-5 (right panel, Student’s *t*-test, *P* < 0.05).

**Supplemental Fig. 3 Cancer stem cell properties of K19+ PLC/PRF/5 and Hep3B cells *in vivo*.** (A) Tumors produced by K19+ and K19− cells. (B) Sequential tumor size generated from K19+ or K19− cells in NOD/SCID mice (repeated-measures ANOVA, *P* < 0.01). Data are shown as the mean ± SD (K19+ PLC/PRF/5: n = 9, K19− PLC/PRF/5: n = 5, K19+ Hep3B: n = 9, K19− Hep3B: n = 3). (C) Hematoxylin-eosin and K19 staining of tumors. Scale bar represents 100 µm. (D) FACS analyses of initial and serial transplantation. In initial transplantation, tumors were generated from 1 × 104 sorted K19+ (right panel) or K19− (left panel) cells derived from a single K19+ cell. In serial transplantation, tumors were generated from sorted 1 × 104 K19+ (right panel) or K19− (left panel) cells derived from tumors produced by K19+ cells.