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JIB-04, a Pan-Inhibitor of Histone Demethylases, Targets Histone-Lysine-Demethylase-Dependent AKT Pathway, Leading to Cell Cycle Arrest and Inhibition of Cancer Stem-Like Cell Properties in Hepatocellular Carcinoma Cells

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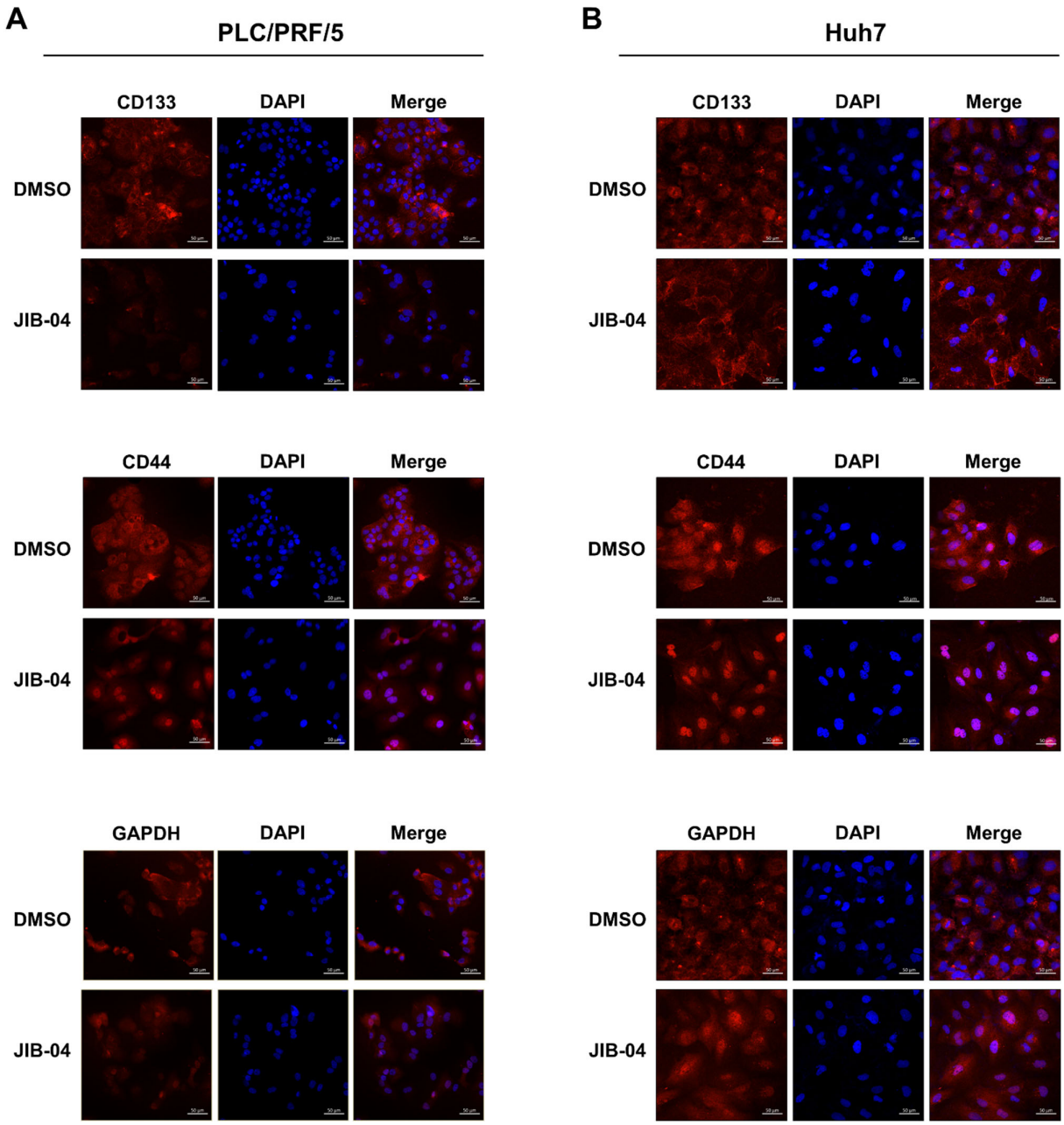
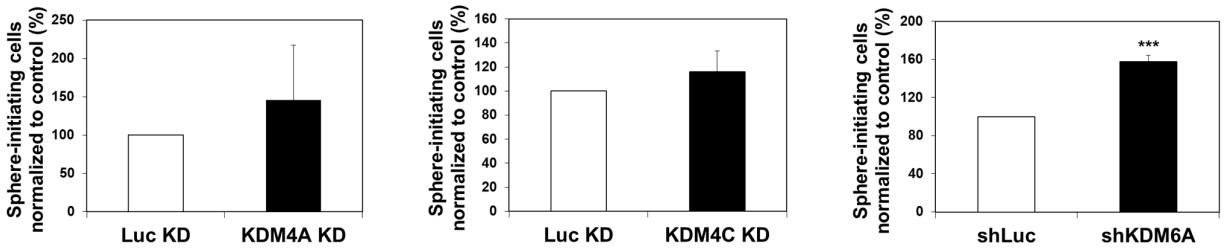
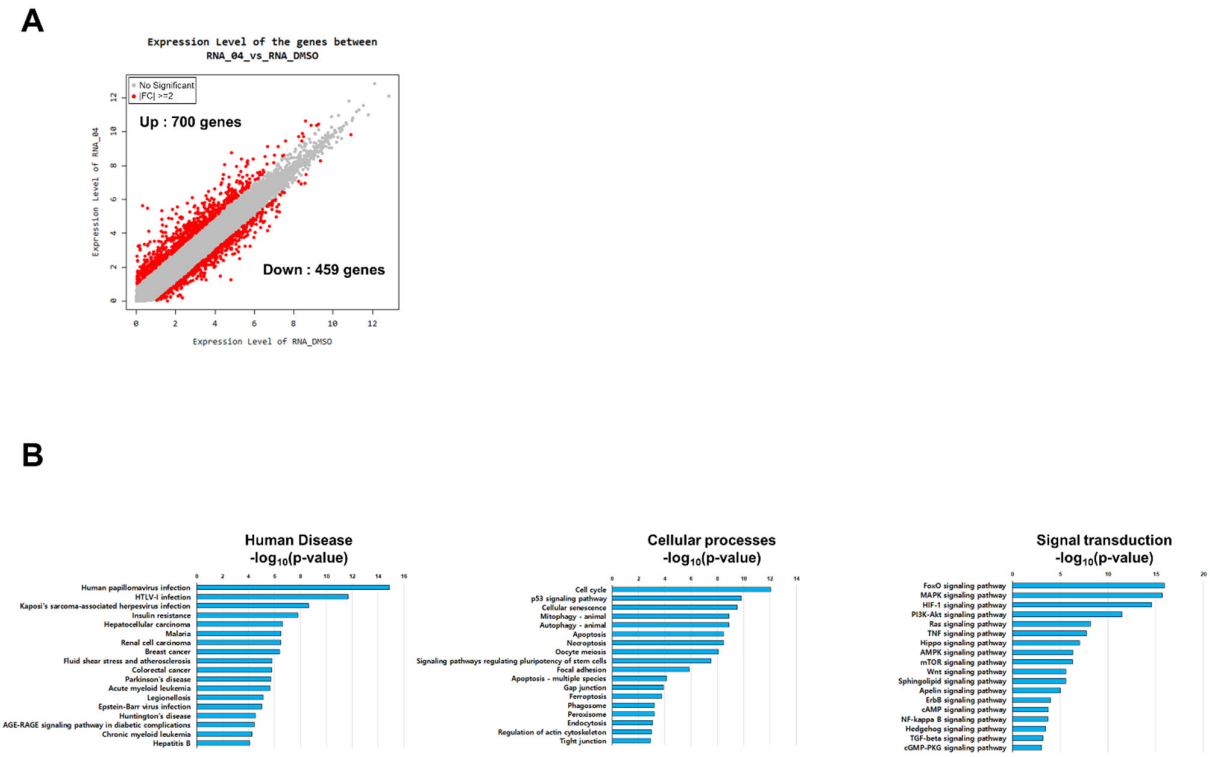


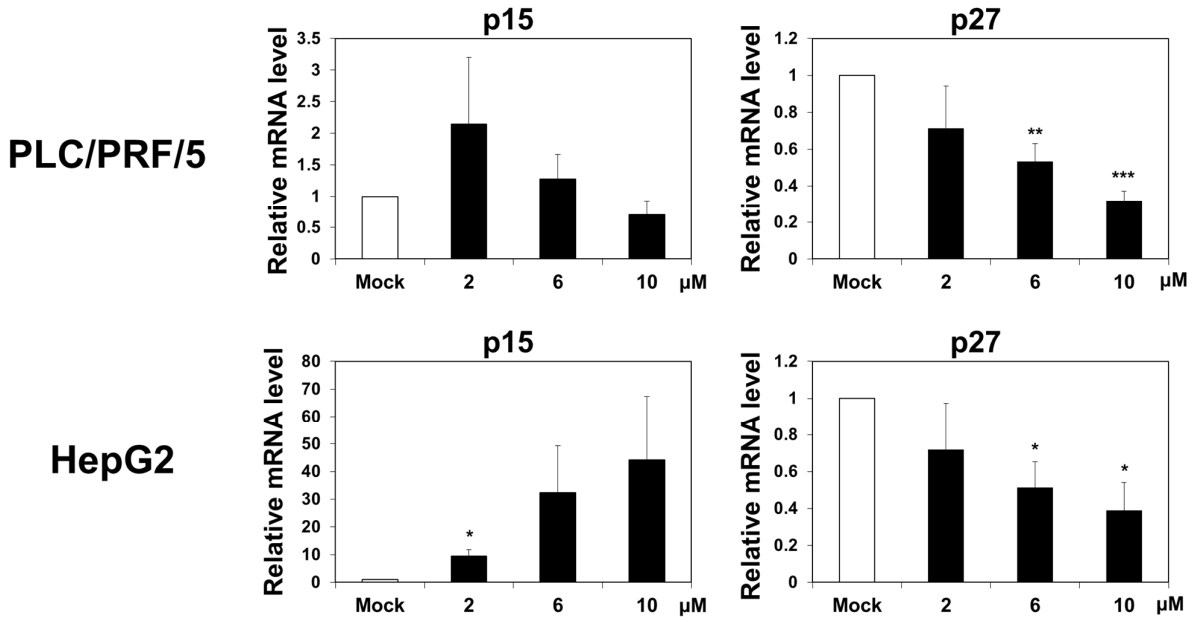
Supplementary Figure S1. The expression of cancer stem cell markers was decreased in JIB-04-treated HCC Cells. The fluorescent immunocytochemistry experiment showed that the expression of CD133 and CD44 was decreased in PLC/PRF/5 cells (A) and Huh7 cells (B). Representative images from two independent experiments are shown. Scale bar: 50 μM.



Supplementary Figure S2. KDM4A, KDM4C, and KDM6A are not required for the tumorsphere formation ability of CSCs in PLC/PRF/5 cells. CCK assay revealed that the viability of tumorsphere cells was increased in *KDM4A*-, *KDM4C*-, and *KDM6A*-depleted knockdown cells (n=3). Percentage of sphere-initiating cells measured by CCK assay. The number of control-knockdown cells (Luc KD) was set as 100. Data are represented as mean ± SEM of triplicate measurements. ***p <0.001.



Supplementary Figure S3. Transcriptome analysis of PLC/PRF/5 cells after treatment with JIB-04. (A) Scatter plot of differential gene expression between cells treated with 6 μM JIB-04 or DMSO. (B) KEGG pathway enrichment analysis. Raw *p*-values were calculated by modified Fisher's exact test. The pathway enrichment scores (−Log10 *P*-value) among the differentially expressed genes are shown.



Supplementary Figure S4. The effect of JIB-04 treatment on the mRNA expression of CDK inhibitors such as *p15(Ink4b)* and *p27(kip1)*. The mRNA expression levels of *p15(Ink4b)* and *p27(kip1)* in PLC/PRF/5 and HepG2 cells cultured after treatment with increasing doses of JIB-04 or DMSO (mock control) were measured by qRT-PCR. All data are normalized to GAPDH and plotted relative to the expression level in control cells (n=3). Data are represented as mean ± SEM of triplicate measurements. *p <0.05, **p <0.01, and ***p <0.001.