Figure S1

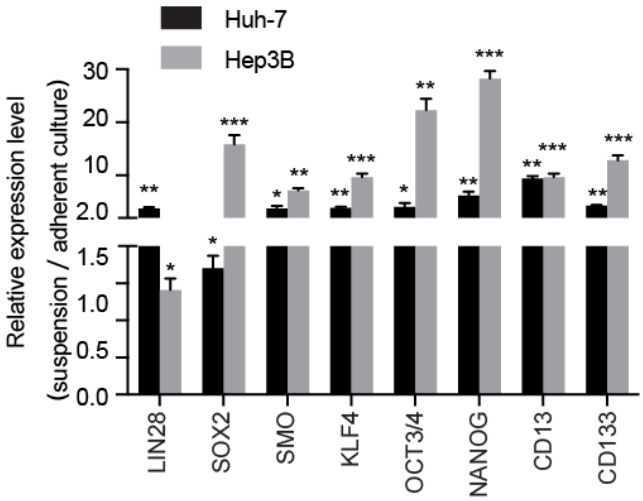

Figure S1. Liver cancer stem cells (LCSCs) were enriched by spheroid formation assay. Real-time PCR analysis of the expression of stemness markers, including LIN28, SOX2, SMO, OCT3/4, KLF4, NANOG and CD13, and LCSC marker CD133. The fold changes of mRNA expression represented tumor spheres versus the adherent Hep3B cells. The mRNA expression levels were normalized against β-actin. The data are presented as the mean ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure S2

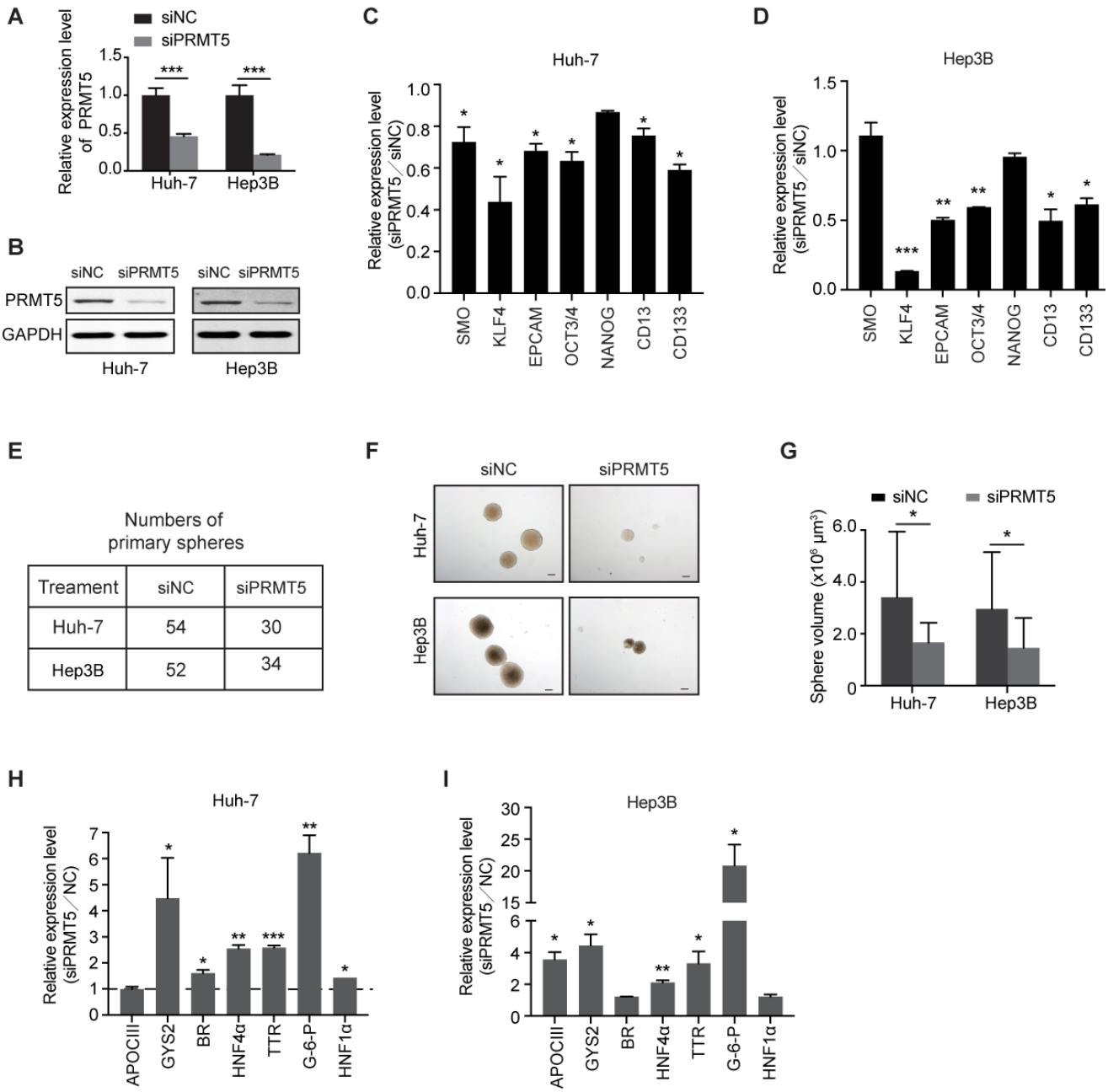

Figure S2. Knockdown of PRMT5 downregulates the expression of stemness markers and upregulates the expression of characteristic hepatocyte markers.

(A, B) Huh-7 and Hep3B cells were transfected with control siNC and siPRMT5. The expression of PRMT5 was evaluated by real-time PCR and western blotting analysis. (C, D) The expression of cancer stemness markers in Huh-7 (C) and Hep3B (D) cells was detected by

real-time PCR. (E) Huh-7 and Hep3B cells transfected with control siNC and siPRMT5 were assessed for spheroid formation assay. The frequency of primary spheres was showed. (F) Representative photographs of tumor spheres of siNC and siPRMT5 in HCC cells. Scale bars = 100 μm. (G) Quantification analysis of the size of tumor spheres formed from siNC and siPRMT5. (H, I) The expression of liver specific genes in Huh-7 (H) and Hep3B (I) cells was detected by real-time PCR. The data are presented as the mean ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure S3

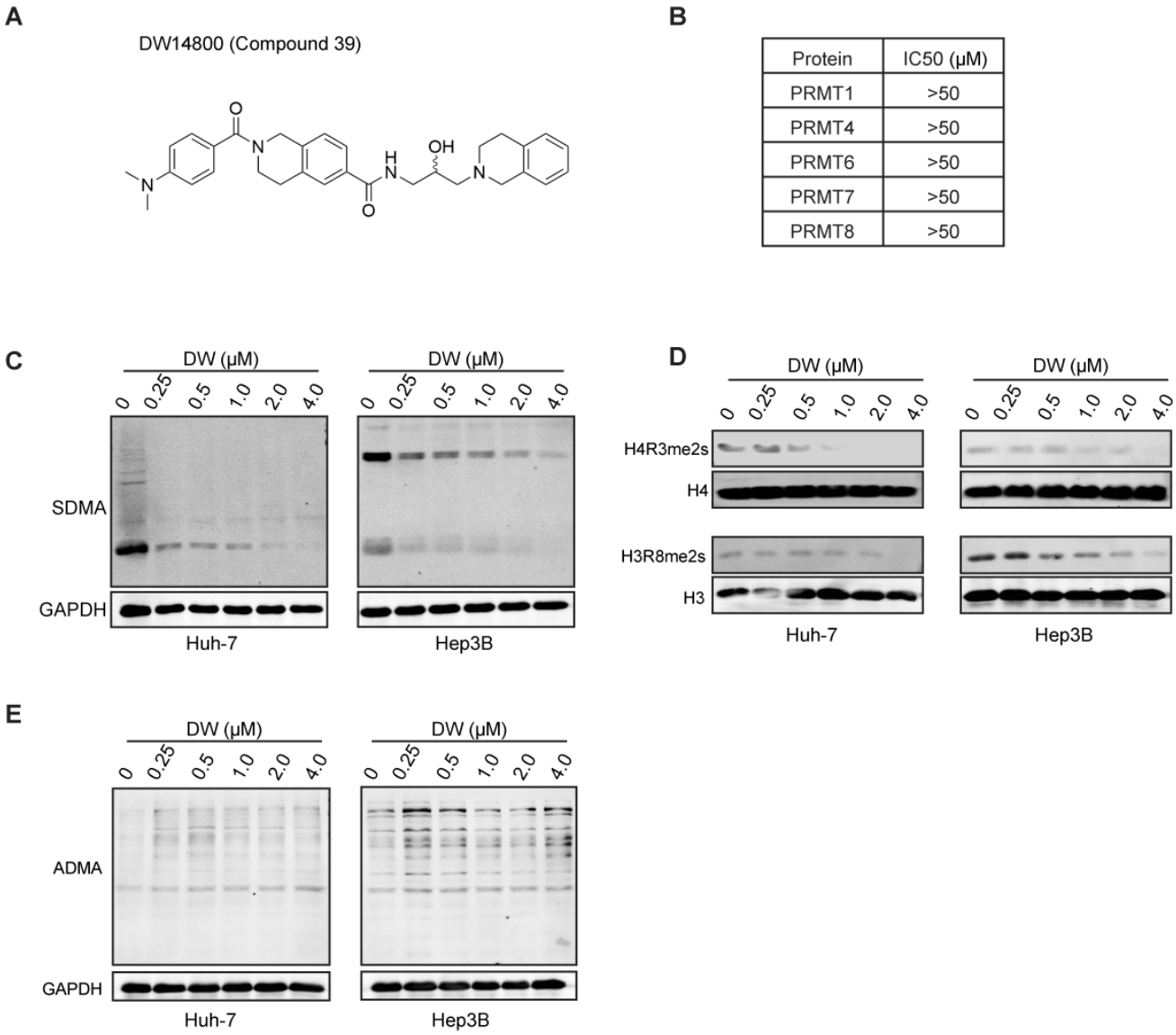

Figure S3. The novel PRMT5 inhibitor DW14800 selectively reduced the cellular symmetric dimethylarginine.

(A) Structure of DW14800. (B) Selectivity profile of DW14800. DW14800 showed no activity against the other PRMT enzymes at a 50 μM concentration. (C) The effects of DW14800 on symmetric dimethylarginine (SDMA) in Huh-7 and Hep3B cells. (D) The effects of DW14800 on symmetric dimethylation of histone H4 on arginine 3 (H4R3me2s) and histone H3 on arginine 8 (H3R8me2s) in Huh-7 and Hep3B cells. (E) Effects of

DW14800 on asymmetric dimethylarginine (ADMA) in Huh-7 and Hep3B cells were detected by antibody against Asymmetric Di-Methyl Arginine Motif.

Figure S4

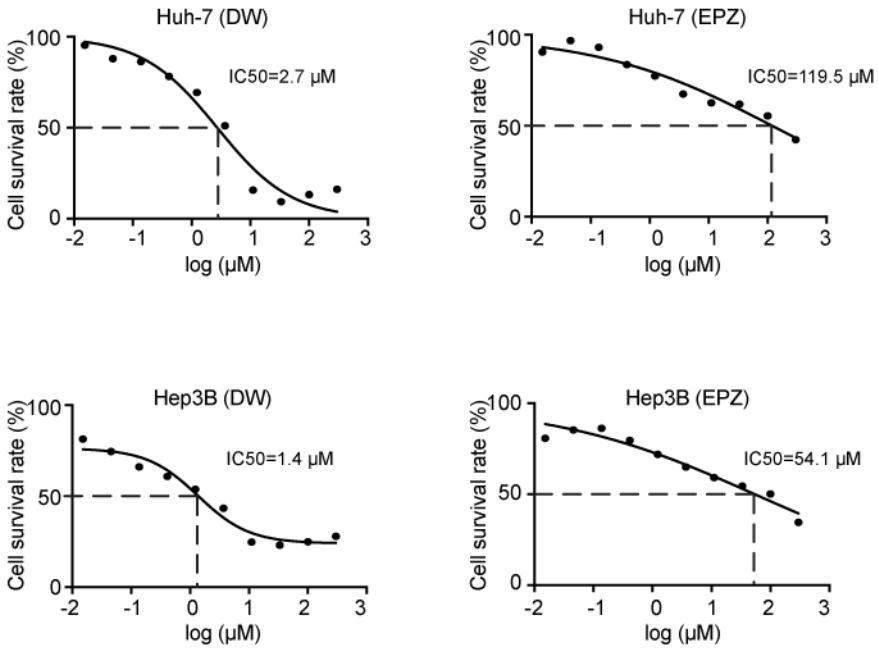

Figure S4. The IC50 values of DW14800 were dramatically lower than EPZ015666.

HCC cells were treated with DW14800 or the reported inhibitor EPZ015666 for 3 days. The viability of the cells was detected with CCK8.

Figure S5

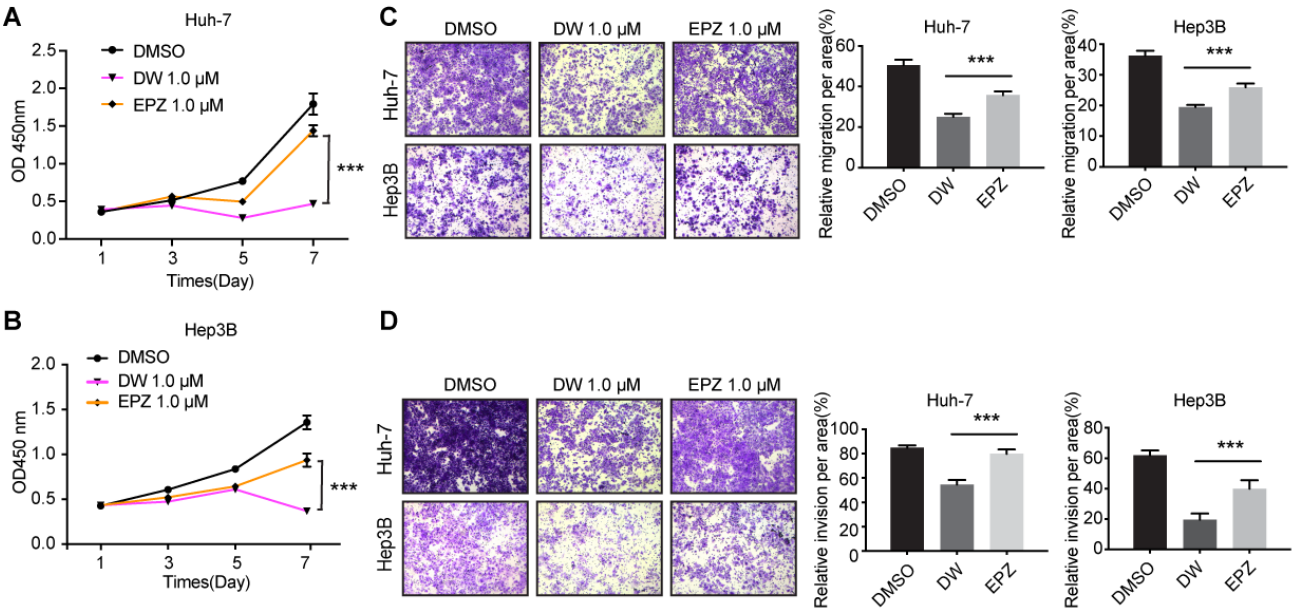

Figure S5. The effects of DW14800 and EPZ015666 on HCC malignancy.

Huh-7 and Hep3B cells were treated with DW14800 and EPZ015666 at indicated concentration and the cell proliferation (A, B), migration (C) and invasion (D) were examined. The data are presented as the mean ± SD. *** $P < 0.001$.

Figure S6

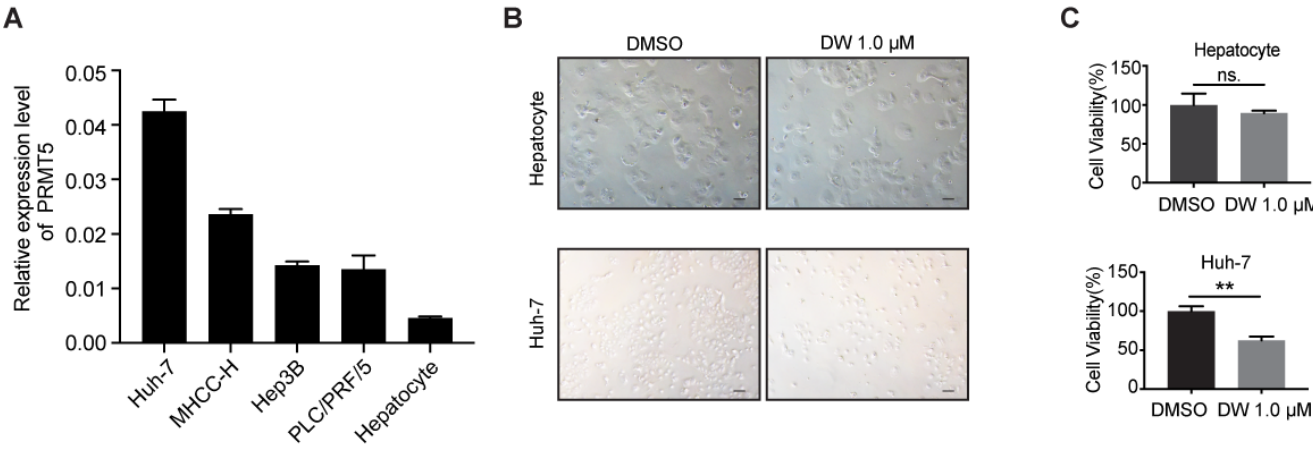

Figure S6. Cytotoxic effect of DW14800 on primary human hepatocytes and Huh-7 cells.

(A) The relative expression levels of PRMT5 in representative HCC cell lines and primary human hepatocytes. (B) The morphology of primary human hepatocytes and Huh-7 cells treated with DW14800 for 5 days. Scale bars = 100 μm. (C) The cell viability was measured by CCK8 assay. The data are presented as the mean ± SD. ** $P < 0.01$, ns. no statistical significance.

Table S1. PCR primers used in this study

Genes	Forward primer (5'- 3')	Reverse primer (5'- 3')
β-actin	CATCCTGCGTCTGGACCT	GTACTTGCGCTCAGGAGGAG
LIN28	TGTAAGTGGTTCAACGTGCG	CCTCACCCTCCTTCAAGCTC
CD133	CATCTCTCAATGACCCTCTGTG	CCTCAGTTCAGGGTTGCTATT
CD13	GTTCTCCTTCTCCAACCTCATC	CTGTTTCCTCGTTGTCCTTCT
SOX2	GCGAACCATCTCTGTGGTCT	GGAAAGTTGGGATCGAACAA
SMO	ATCTCCACAGGAGAGACTGGTTCGG	AAAGTGGGGCCTTGGGAACATG
KLF4	GCGGCAAAACCTACACAAAG	CCCCGTGTGTTTACGGTAGT
OCT3/4	CGACCATCTGCCGCTTTGAG	CCCCCTGTCCCCCATTCCTA
NANOG	GATTTGTGGGCCTGAAGAAA	TTGGGACTGGTGGAAGAATC
PRMT1	CAGGCGGAAAGCAGTGAGAA	TGGAGTTGCGGTAAGTGAGG
PRMT5	TTGCCGGCTACTTTGAGACT	ACAGATGGTTTGGCCTTCAC
EHMT2	GGAGGAAGAGGAGGAAGAAGAA	CCACTGGAACCACTCCTATCT
SETD8	CATGAAGTCCGAGGAACAGAAG	GAATCACAAGATGAGGGTGGAG
DNMT1	CGGCCTCATCGAGAAGAATATC	AAGCCAGTGATCCACCATTC
DNMT3B	GATGAAGATCAGAGCCGAGAAC	TCAAAGAGAGGGTGGAAGGA
HNF4α	CTTCCTTCTTCATGCCAG	ACACGTCCCCATCTGAAG
APOCIII	GGGTACTCCTTGTTGTTGC	AAATCCCAGAACTCAGAGAAC
GYS2	CCAGTGGGAAGTCGAAGAAC	TTCTCTCCCCATTCATCTGC
BR	ACAAGGTGCTGCGGGAATCA	ACTGGTGGGAGGGGTAGGTG
TTR	TCAGAAAGGCTGCTGATGAC	AGTCGTTGGCTGTGAATACC
ALDOB	AGGAGGACTCTTCTCTCCCAA	GATTCATCTGCAGCCAGGAT
G-6-P	GGCTCCATGACTGTGGGATC	TTCAGCTGCACAGCCCAGAA
HNF1α	TGATGAGCTACCAACCAAGAAG	TAGGGTTCTTCTGCCTCTCATA

Table S2. PCR primers for ChIP used in this study

HNF4α-a	CCGTTAAGCAATGTGGAAC	GAAGAGATGTGGAGGGCAG
HNF4α-b	GTATTTGGTCTCTTTCAGC	TTATTTACATCGTCTCTTC
HNF4α-c	AGGCAATGAGGGCTGGAGG	AGAGTGGGCTTTGTGGGGG
HNF4α-d	ATGCCATCTCATTTCCTTC	TTTTCCCATCACATTCTCC
HNF4α-nc	TGCACAGTAGGTGTGATGCTGA	AACCATGTGACCGTGTTCAGTC
eIF4E	TGGTAGAATCTCAAATAGGCAAAG	ACAGAGTTTCAGGAAAATCTCCA