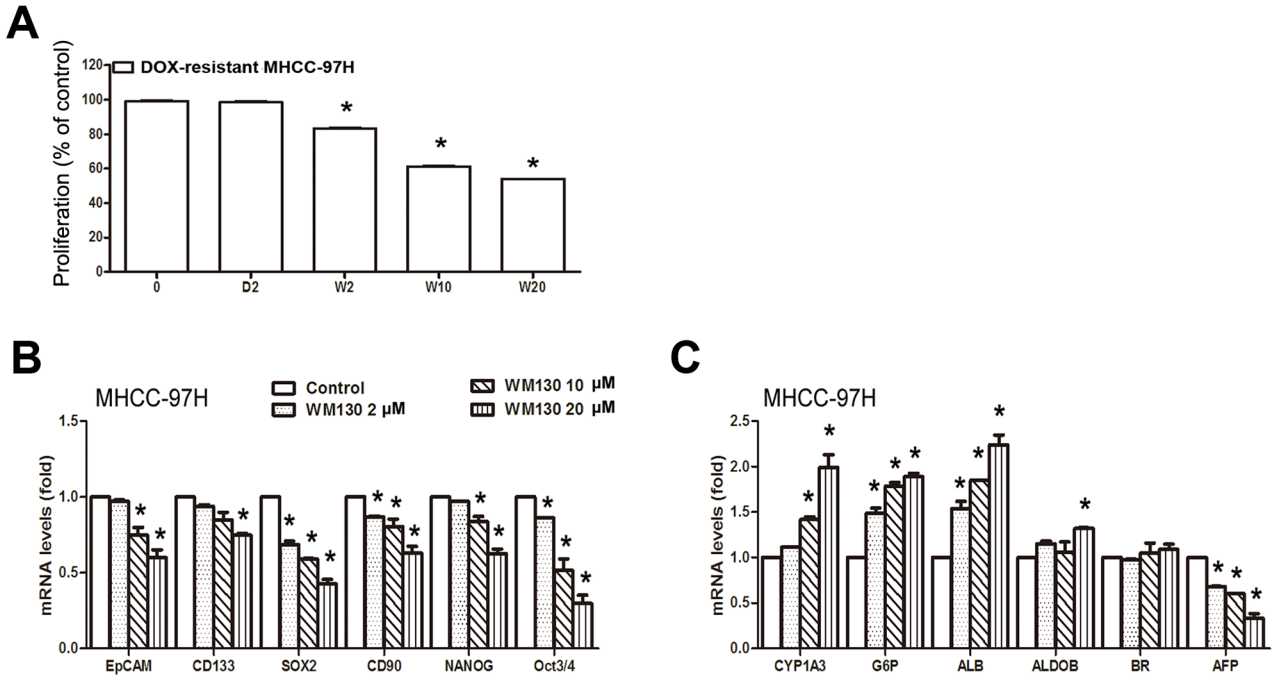
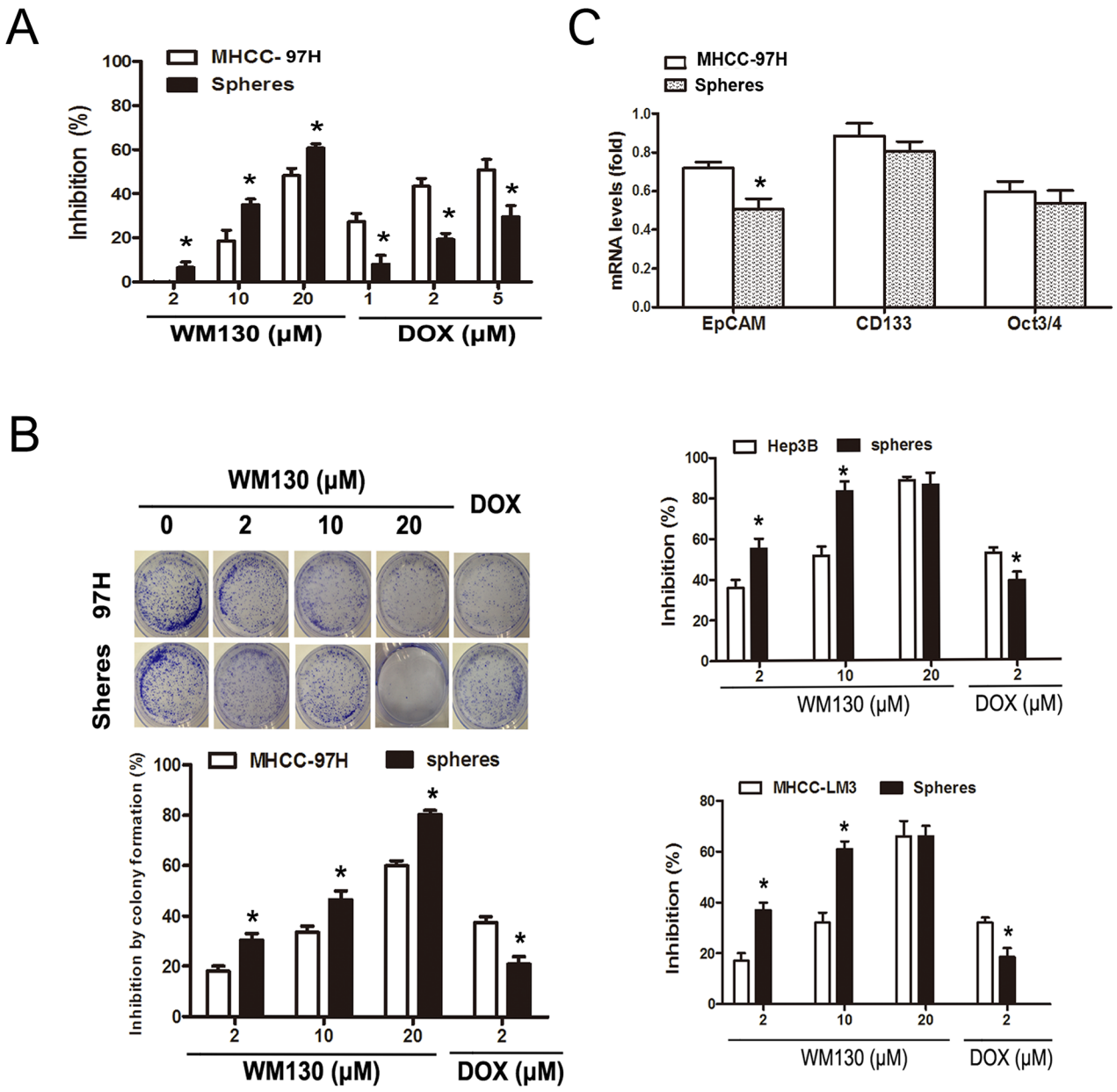
WM130 preferentially inhibits hepatic cancer stem-like cells by suppressing AKT/GSK3β/β-catenin signaling pathway

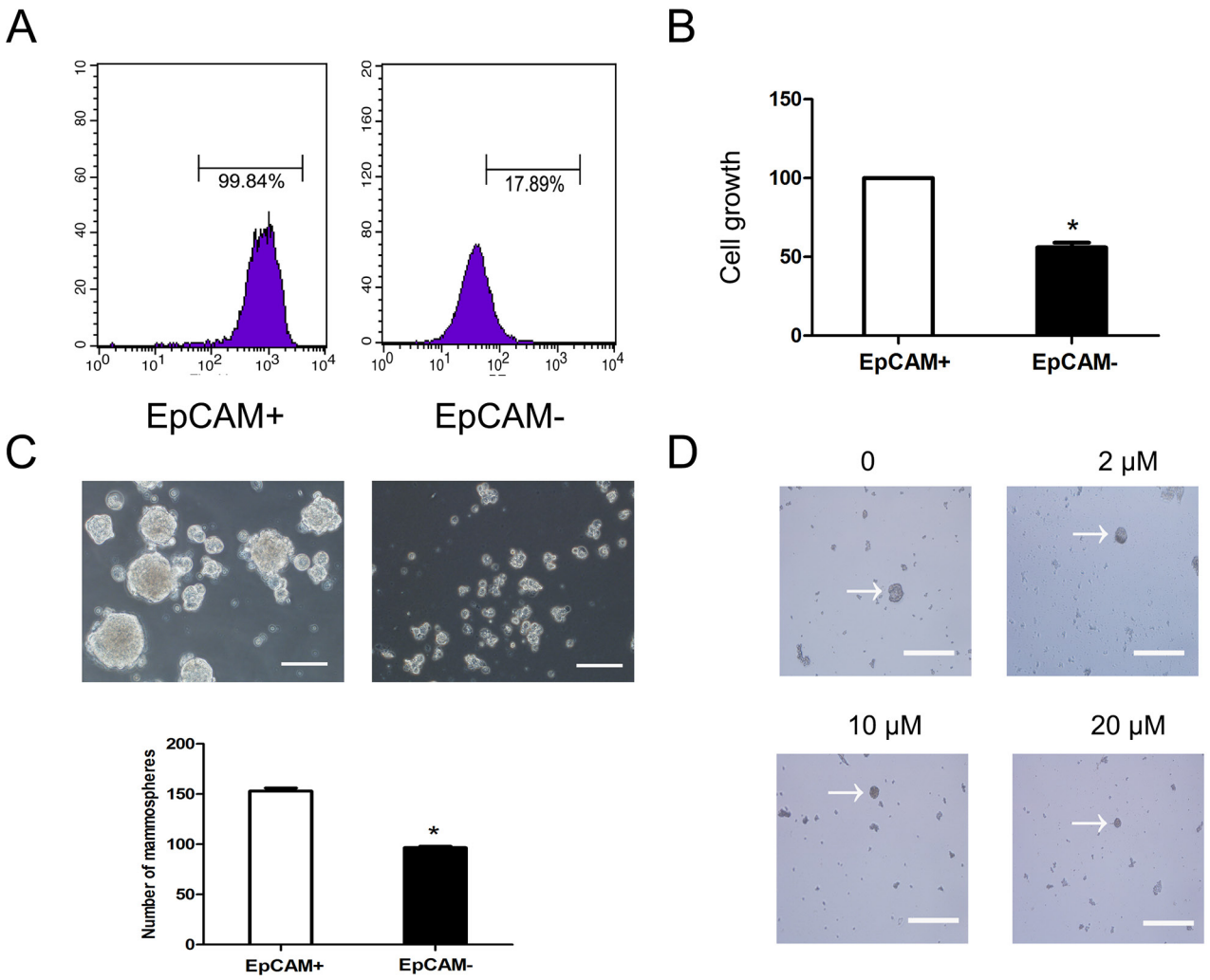
SUPPLEMENTARY FIGURES AND TABLE



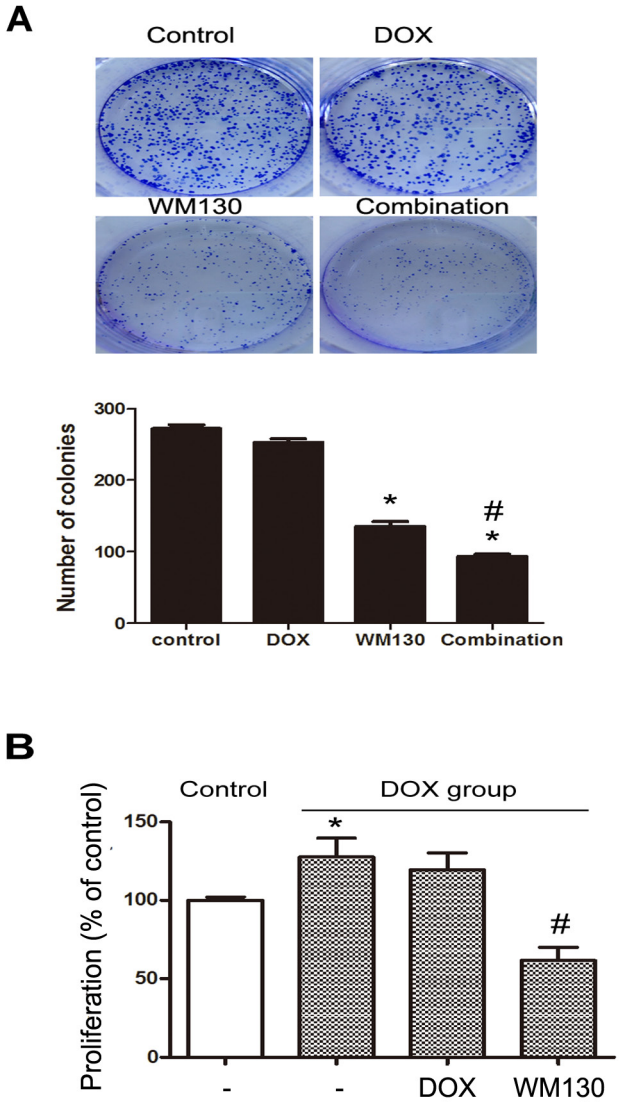
Supplementary Figure S1: The effect of WM130 on DOX-resistant MHCC-97H cells and the expression of genes relevant to stemness and differentiation of hepatic stem/progenitor cells. A. DOX-resistant MHCC-97H cells remained sensitive to WM130. N=3, *p<0.05 versus control. **B** and **C.** MHCC-97H cells were treated with WM130 for 24 h. The expression of "stemness" and liver-specific genes was detected by real-time RT-PCR. The mRNA levels were normalized against β-actin and are relative to the control. N=3, *p<0.05 versus control.



Supplementary Figure S2: Preferential inhibition of MHCC-97H sphere cells by WM130. A. Growth inhibitory effects of WM130 and DOX on MHCC-97H sphere cells compared with their corresponding parental cells. N=3, **p*<0.05 versus control. **B.** Inhibition of colony formation by WM130 and DOX. N=3, **p*<0.05 versus control. **C.** WM130 preferentially decreased EpCAM expression in spheroids. N=3, **p*<0.05 versus control.



Supplementary Figure S3: The sorted EpCAM+ Hep3B cells exhibits features of cancer stem/progenitor cells. A. Purity of the sorted of $EpCAM^+$ and $EpCAM^-$ Hep3B cells. **B.** The sorted $EpCAM^+$ Hep3B cells exhibited stronger proliferation ability than $EpCAM^-$ cells. N=3, *p<0.05 versus $EpCAM^+$ Hep3B cells. **C.** The sorted $EpCAM^+$ Hep3B cells exhibited better sphere forming ability than $EpCAM^-$ cells. N=3, *p<0.05 versus $EpCAM^+$ Hep3B cells. Scale bar=100 μM. **D.** WM130 noticeably reduced the size of single-cell derived $EpCAM^+$ monoclones in a concentration dependant manner. Scale bar=100 μM.



Supplementary Figure S4: Colony formation and proliferation of cancer cells isolated from dissociated MHCC-LM3 tumors. A. Colony formation of cancer cells isolated from dissociated MHCC-LM3 tumors. N=6, *p<0.05 versus control and $^{\#}$P<0.05 versus groups treated with either agent alone. **B.** Cancer cells from DOX-treated mice showed higher proliferation compared with cells from control-treated mice and were *in vitro* resistant to DOX (2 μmol/L) but sensitive to WM130 (10 μmol/L) as determined by CCK8 assays. N=3, *p<0.05 versus control-treated mice and $^{\#}$ p<0.05 versus untreated control.

Supplementary Table S1: Sequence of primers

Gene	Sequence
β-actin	F: 5′-ACCCACACTGTGCCCATCTATG-3′ R: 5′-AGAGTACTTGCGCTCAGGAGGA-3′
EpCAM	F: 5’-GCTCTGAGCGAGTGAGAACCT-3’
	R: 5’-GACCAGGATCCAGATCCAGTTG-5’
CD133	F: 5′-ACATGAAAAGACCTGGGGG-3′ R: 5′-GATCTGGTGTCCCAGCATG-3′
CD90	F: 5′-CGGAAGACCCCAGTCCA-3′ R: 5′-ACGAAGGCTCTGGTCCACTA-3′
G-6-P	F: 5′-GGCTCCATGACTGTGGGATC-3′ R: 5′-TTCAGCTGCACAGCCCAGAA-3′
ALB	F: 5′-AGCCTAAGGCAGCTTGACTT-3′ R: 5′-CTCGATGAACTTCGGGATGA -3′
BR	F: 5′-ACAAGGTGCTGCGGGAATCA-3’ R: 5′-ACTGGTGGGAGGGGTAGGTG-3’
CYP1A3	F: 5′-CTGGCCTCTGCCATCTTCTG-3′ R: 5′-TTAGCCTCCTTGCTCACATGC-3′
Oct3/4	F: 5′-CGACCATCTGCCGCTTTGAG-3′ R: 5′-CCCCCTGTCCCCCATTCCTA-3′
Nanog	F: 5’-CCCTGATTCTCCCACCAGTCC-3’
	R: 3’-AGTCGGGTTCACCAGGCATCC-3’
Sox2	F: 5′-GCGAACCATCTCTGTGGTCT-3′ R: 5′-GGAAAGTTGGGATCGAACAA-3′
AFP	F: 5’-TACGTCCCTCCACCATTTC-3’ R: 5’-ATCCTGGTCTTTGCAGCACT-3’