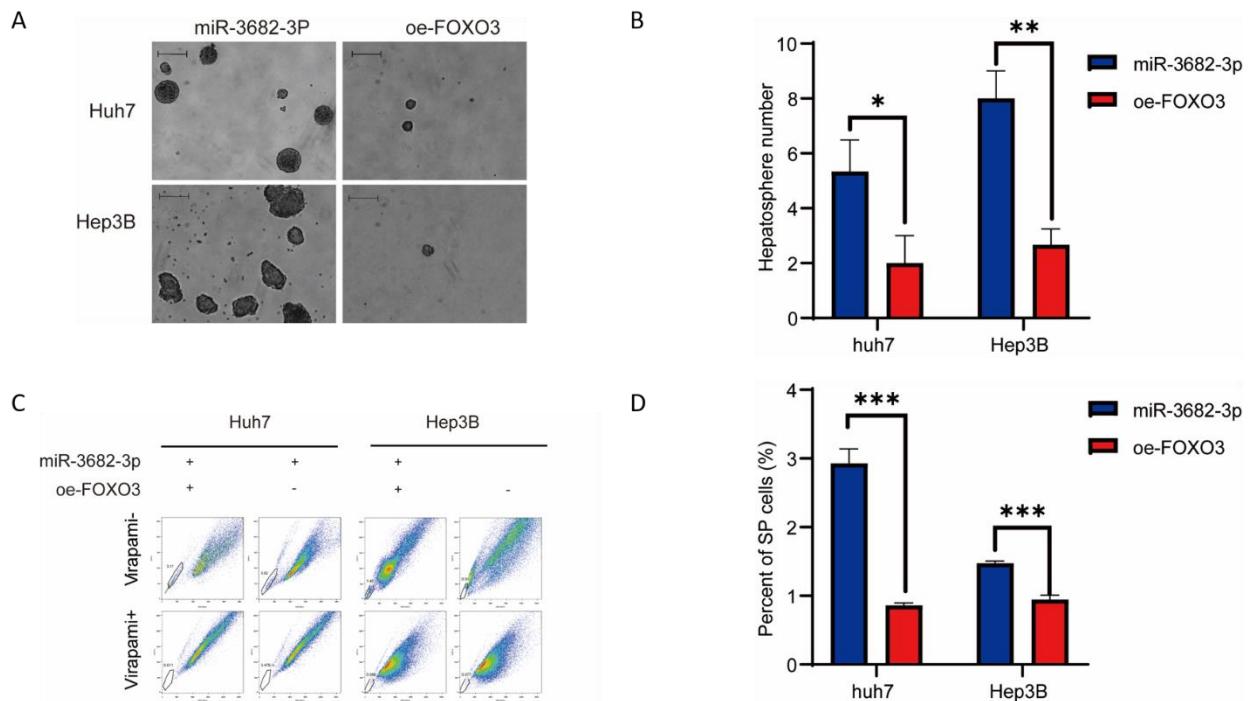


Supplementary Figure 1 miR-3682-3p knockdown suppresses stemness in hepatocellular carcinoma cells. A: MiR-3682-3p expression was examined in hepatocellular carcinoma (HCC) cells stably overexpressing miR-3682-3p and control cells (scale bar: 200 μ m); B: miR-3682-3p expression in HCC cells treated with miR-3682-3p mimic and control cells; C: Statistical analysis of the numbers of hepatospheres formed; D: Statistical analysis of the percentage of side population (SP) cells; E: A DCV dye exclusion assay showed that silencing miR-3682-3p suppressed the proportions of SP cells among the indicated cells; F: Statistical analysis of the percentage of SP cells; G: RT-qPCR analysis of SOX2, OCT4, CD133, and CD44 expression; H: Western blot-based detection of changes in the levels of stemness markers after miR-3682-3p silencing. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Experiments were repeated three times. NC: Negative control.



Supplementary Figure 2 FOXO3 is a direct target gene of miR-3682-3p in hepatocellular carcinoma. A: Representative images and quantification of the numbers of hepatospheres formed by the indicated cells (scale bar: 200 μ m); B: Statistical analysis of the numbers of hepatosphere formed; C: A DCV dye exclusion assay showed that FOXO3 overexpression led to a reduction in the proportions of side population (SP) cells among the indicated cells; D: Statistical analysis of the percentage of SP cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Experiments were repeated three times.

Supplementary Table 1 The primers used in this study

Primers name		Sequence (5'-3')
FOXO3	Forward	GGCAAAGCAGACCCTCAAAC
	Reverse	TGTCCACTGCTGAGAGCAG
c-Myc	Forward	GCTGCTTAGACGCTGGATT
	Reverse	TAACGTTGAGGGGCATCG
HBX	Forward	CACTTCGCTTCACCTCTGC
	Reverse	TCGGTCGTTGACATTGCTG
GAPDH	Forward	CTAAGGCCAACCGTGAAAAG
	Reverse	ACCAGAGGCATAACAGGGACA
OCT4	Forward	AGCGATCAAGCAGCGACTA
	Reverse	GGAAAGGGACCGAGGAGTA
SOX2	Forward	CATCACCCACAGCAAATGAC
	Reverse	CAAAGCTCCTACCGTACCACT
CD44	Forward	GACACCACAGACAGAACATC
	Reverse	AGTAGTTGAGCCTTCAGAA
CD133	Forward	CAACGAGTCCTCCTATA
	Reverse	CTCTCCAACAATCCATTTC
Chip-c-Myc-A	Forward	CTGTCATGCATTGGCAATATTAA
	Reverse	CTTGAATACTGTCAGGTGGTTAGT
Chip-c-Myc-B	Forward	CCTCCAGAGTAGCTGGGATTA
	Reverse	CATGGTGAAACCCTGTCTCTAC
Chip-c-Myc-C	Forward	TTTGAGTTGAGATGATTGACTGG
	Reverse	TGATGAGCATAAAGAGACAATACCT
miR-3682-3p	Forward	TGATGATACAGGTGGAGGTAGGT

Supplementary Table 2 The sequences used in this study

Gene	NO.	Target Seq
FOXO3	1	GAAGTATGCTGTTCTCTAT
	2	CCAACAGGATACTTGAAA
	3	GACAGTCGTACTGTGACT
<i>c-Myc</i>	1	CAGAAATGTCCTGAGCAAT
	2	GGTCAGAGTCTGTATCACC
	3	GATGAGGAAGAAATCGATG
HBX	1	GCACUUCGCUUCACCUCUG
	2	GGUCUUACAUAAAGAGGGACU
<i>miR-3682-3p mimic</i>		UGAUGAUACAGGUGGGAGGUAG
<i>miR-3682-3p inhibitor</i>		CUACCUCCACCUGUAUCAUCA
<i>MiR-3682 precursor</i>		ATTATTGTTATAAATTGCATGAAATACATAG
		TGGGCTCGATTCATAGCCCCCGTCCGTATCT
		ATATCTCTATATCTATAGAGACATAACTTCT
		TTCTTCGTAAGTTATATATGTCTACTTCTACC
		TGTGTTATCATAATAAAGGTGTCATGATGAT
		ACAGGTGGAGGTAGAAATATATAACTTATC
		TCTTCATAAGGGCTAAAATGAGAACTAAAAA
		GTAATGAGAATTCAAGTTGAATATGATTAATT
		ATCAGGATATGTTAATTAGTTCTCTGATGC
		TTACTG

Supplementary Table 3 A list of Antibodies used for WB, Co-IP, IF and IHC

Antibodies	Cat. No	Company	Species	Dulution
HBX	Sc-57760	Santa Cruz	Mouse	1:1000(WB)
SOX2	11064-1-AP	Proteintech	Rabbit	1:1000(WB);1:50(IHC);
CD44	156-3C11	Cell Sigaling	Mouse	1:1000(WB);1:50(IF);
c-Myc	10828-1-AP	Proteintech	Rabbit	1:1000(WB)
CD133	18470-1-AP	Proteintech	Rabbit	1:1000(WB);1:50(IF);
OCT4	11263-1-AP	Proteintech	Rabbit	1:1000(WB);1:50(IHC);
β-catenin	L5412	Cell Sigaling	Mouse	1:50(IF);1:50(Co-IP)
β-catenin	51067-2-AP	Proteintech	Rabbit	1:1000(WB)
PI3K	13666S	Cell Sigaling	Mouse	1:1000(WB)
GAPDH	pAb AP0063	Bioworld	Rabbit	1:5000(WB)
PPI3K	E3U1H	Cell Sigaling	Rabbit	1:1000(WB)
AKT	C67E7	Cell Sigaling	Rabbit	1:1000(WB)
PAKT	S473	Cell Sigaling	Rabbit	1:1000(WB)
FOXO3	D19A7/75D8	Cell Sigaling	Rabbit	1:1000(WB);1:50(IF); 1:50(IHC);1:50(Co-IP)
IgG	BS12478	Bioworld	Mouse	1:500(Co-IP)