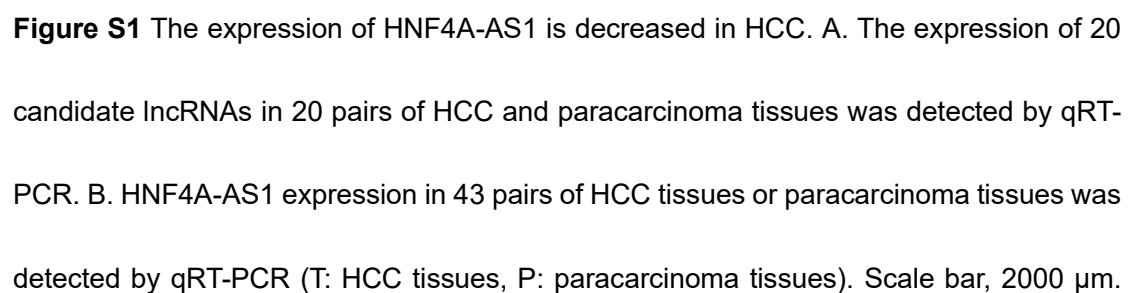


mRNA



Bar graphs represent mean \pm SEM (n=3, *P < 0.05, **P < 0.01, and ***P < 0.001).

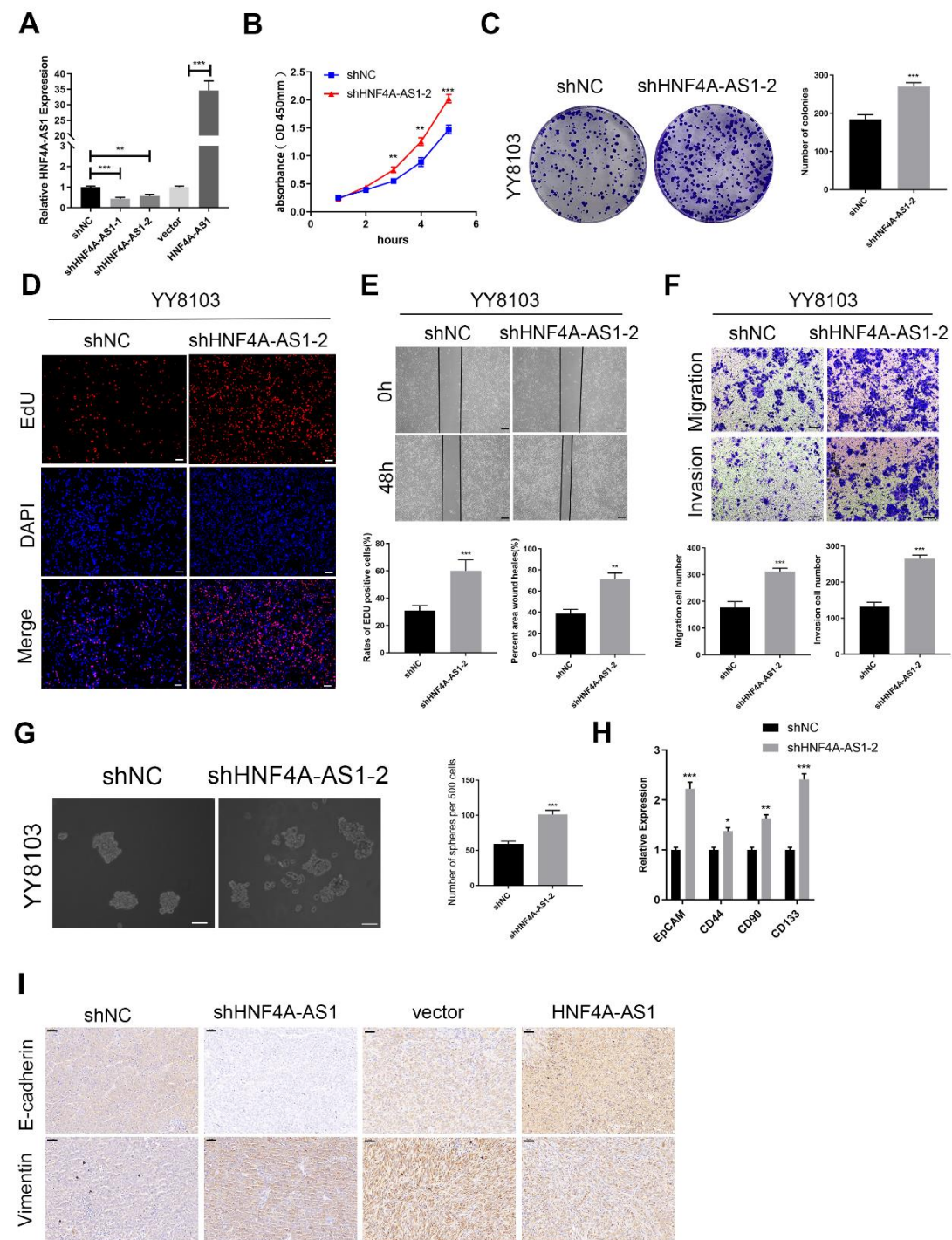


Figure S2 HNF4A-AS1 suppresses proliferation, metastasis and stemness of HCC cells.

A. The qRT-PCR was used to confirm the effectiveness of knocking down and overexpressing HNF4A-AS1. B. The effect of HNF4A-AS1 knockdown on the proliferation of HCC cells was assessed using a CCK8 assay. C-D. A colony formation assay (C) and

EdU assay (D) presented that HNF4A-AS1 knockdown promoted the proliferation of YY8103 cells. Scale bar, 50 μ m. E. A wound-healing assay was used to determine the role of HNF4A-AS1 knockdown in the motility of HCC cells. Scale bar, 500 μ m. F. Transwell assays showed that knockdown of HNF4A-AS1 promoted cell invasion and migration in YY8103 cells. Scale bar, 200 μ m. G. Sphere-forming assays showed that spheroid formation was enhanced in HNF4A-AS1-deficient YY8103 cells. Scale bar, 100 μ m. H. The expression levels of CSC-related biomarkers (EpCAM, CD133, CD44, and CD90) in spheroids were detected by qRT-PCR. I. Immunohistochemistry of E-cadherin and Vimentin in subcutaneous tumors. Scale bar, 50 μ m. Bar graphs represent mean \pm SEM (n=3, *P < 0.05, **P < 0.01, and ***P < 0.001).

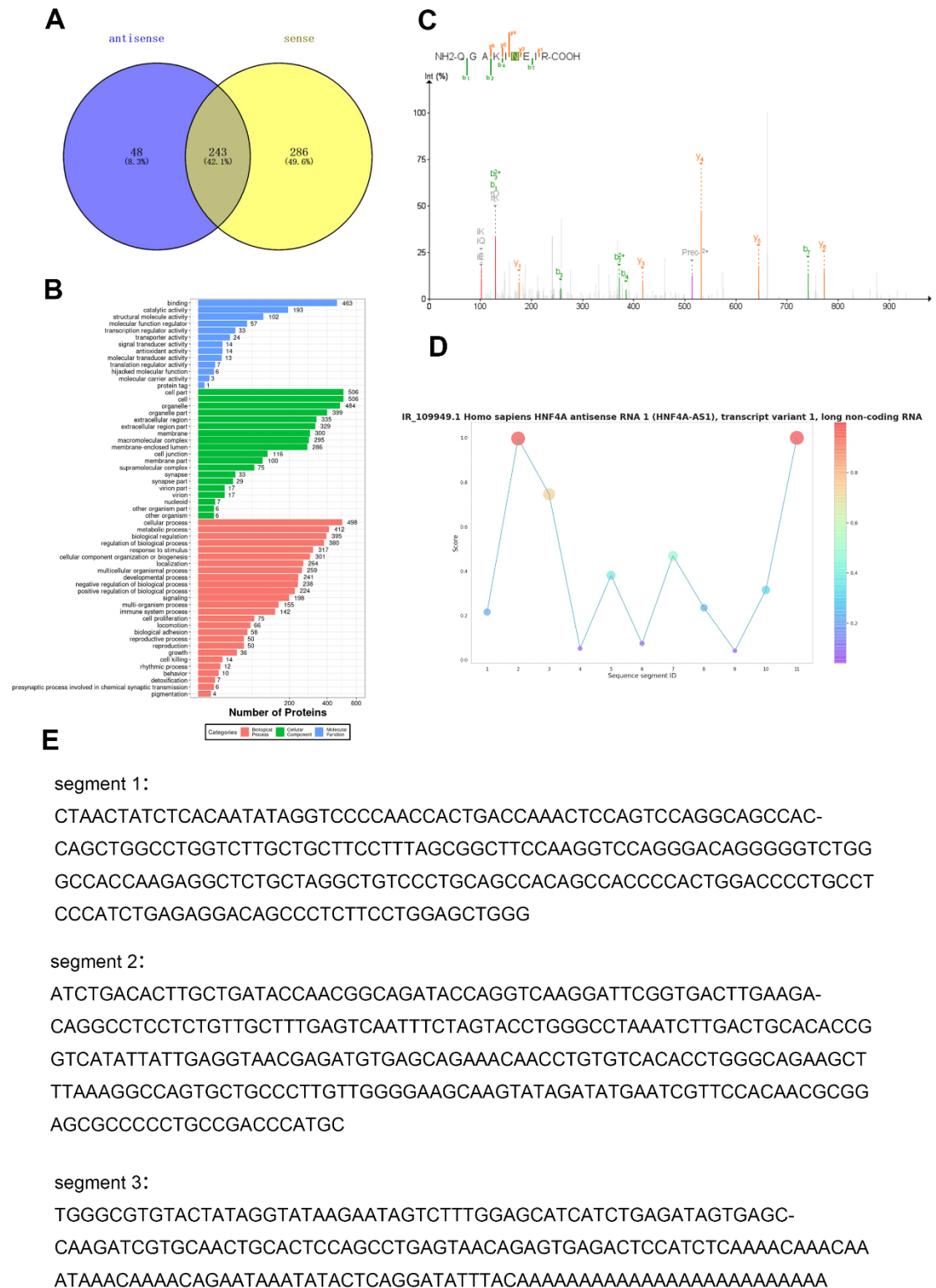


Figure S3 HNF4A-AS1 physically interacts with PCBP2. A. A Venn diagram illustrates the binding proteins of HNF4A-AS1 sense and antisense. B. GO enrichment analysis was conducted on the binding proteins of HNF4A-AS1. C. Mass spectrometry (MS) analysis

was employed to determine the binding of PCBP2 to HNF4A-AS1. D. The score of the PCBP2 binding site of HNF4A-AS1, date from csbio. (<http://www.csbio.sjtu.edu.cn>) E. The deletion mutant sequence of deletion mutants.

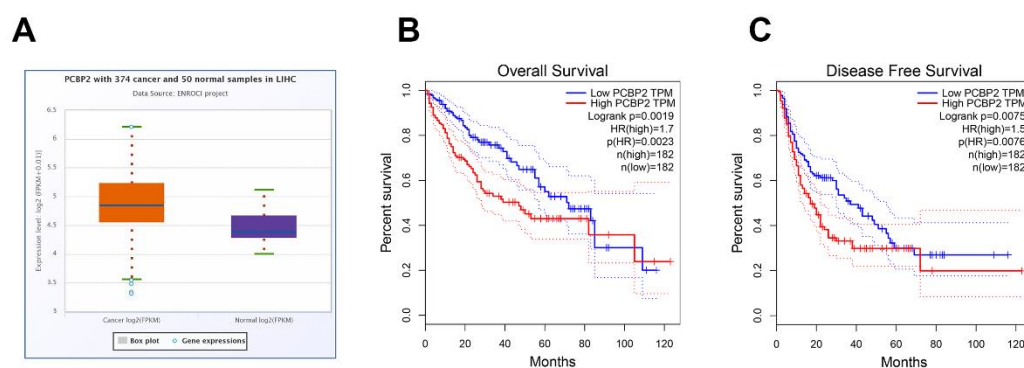


Figure S4 PCBP2 was associated with the prognosis of HCC patients. A. PCBP2 was upregulated in HCC tissues. Data from Starbase. B-C. Overall survival and disease-free survival in HCC patients with high or low expression of PCBP2. Data from GEPIA.

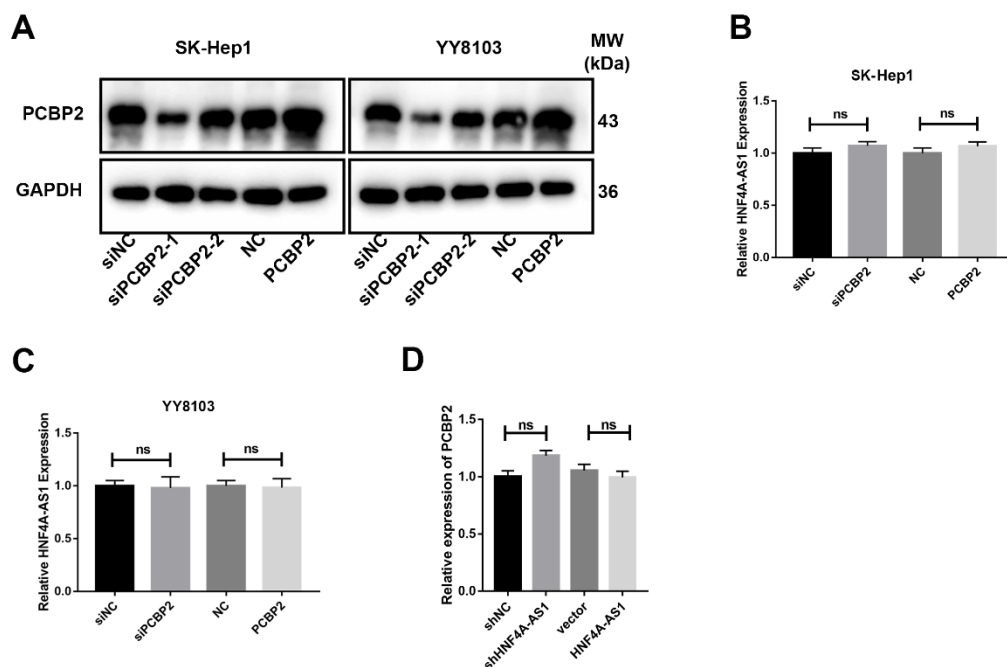


Figure S5 PCBP2 had no regulatory effect on HNF4A-AS1. A. The knockdown and overexpression efficiency of PCBP2 were detected through western blot analysis. B-C. The expression of HNF4A-AS1 was detected by qRT-PCR after PCBP2 knockdown or

overexpression in SK-Hep1 and YY8103 cells. D. qRT-PCR showed that knockdown and levels of HNF4A-AS1 did not affect PCBP2 mRNA expression. Bar graphs represent mean \pm SEM (n=3, *P < 0.05, **P < 0.01, and ***P < 0.001).

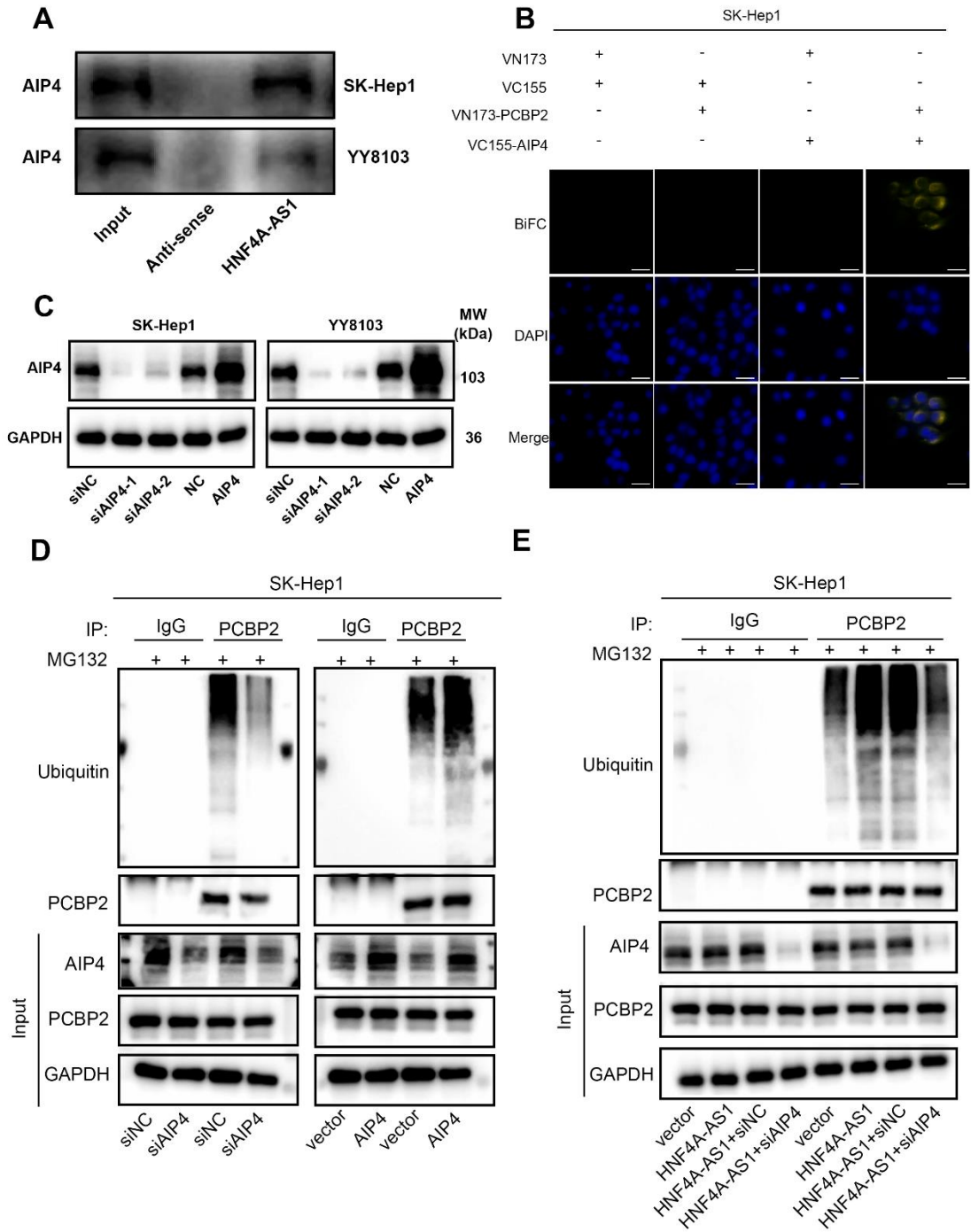


Figure S6 AIP4 was a direct binding partner of HNF4A-AS1. A. Pull-down assays showed that AIP4 was a direct binding partner of HNF4A-AS1. B. The BiFC assay revealed that

AIP4 and PCBP2 were bound in the SK-Hep1 cells. Scale bar, 50 μ m. C. The knockdown and overexpression efficiency of AIP4 was detected through western blot analysis. D. AIP4 knockdown rescued the promoting effect of overexpressed HNF4A-AS1 on PCBP2 ubiquitination. E. Western blot analysis showed that AIP4 promoted PCBP2 ubiquitination in SK-Hep1 cells.

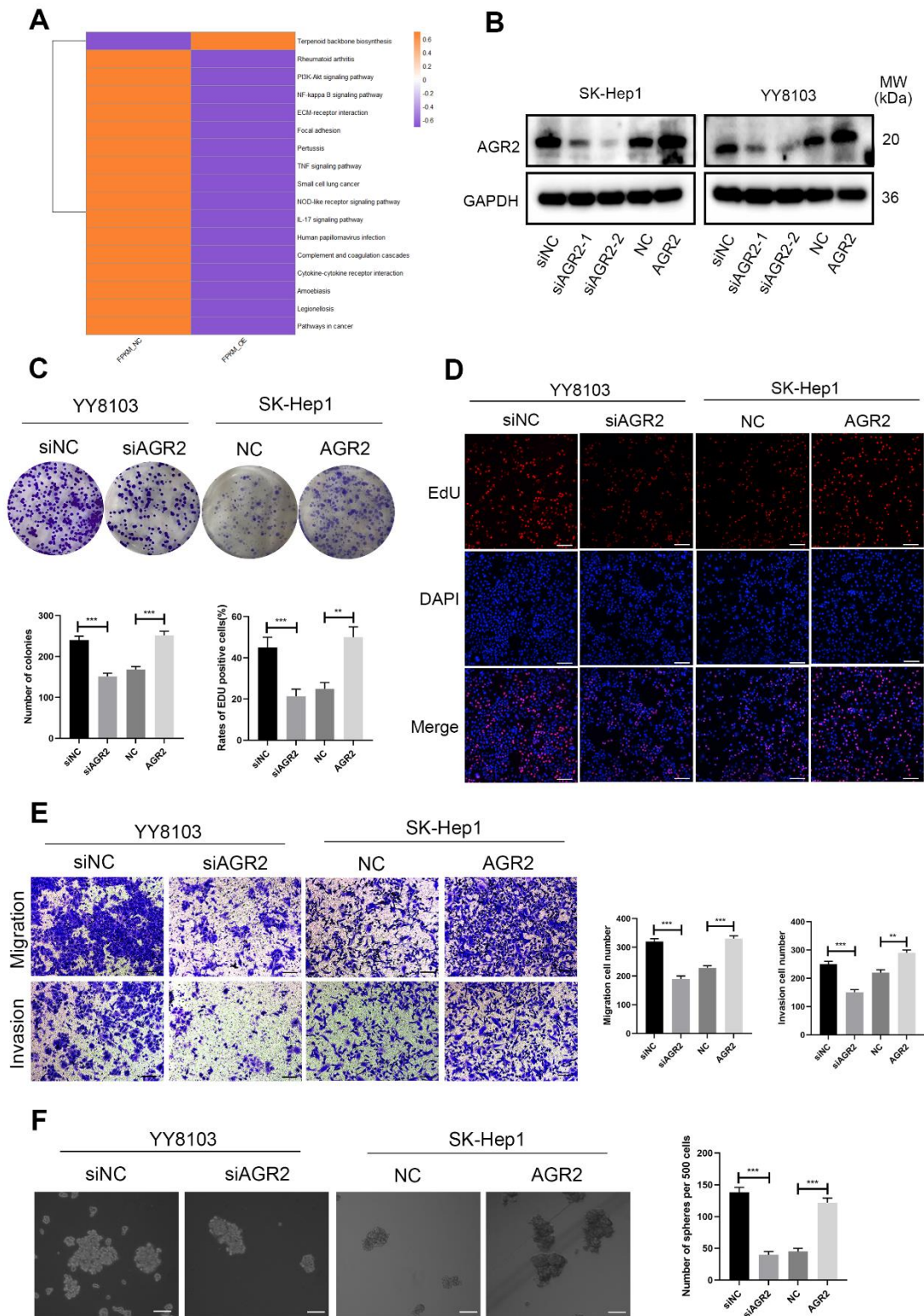


Figure S7 AGR2 promotes proliferation, metastasis, and stemness of HCC cells. A. The heatmap shows the results of KEGG pathway analysis based on RNA-seq data from three pairs of HNF4A-AS1-overexpressing SK-Hep1 cells and negative control cells (NC:

negative control, OE: HNF4A-AS1-overexpressing). B. The knockdown and overexpression efficiency of AGR2 were detected through western blot analysis. C-D. A colony formation assay (C) and EdU assay (D) presented that the overexpression of AGR2 promoted the proliferation of SK-Hep1 cells, while AGR2 knockdown suppressed YY8103 cell proliferation. Scale bar, 50 μ m. E. Transwell assays showed that knockdown of AGR2 inhibited cell invasion and migration in YY8103 cells, while AGR2 overexpression suppressed the migration and invasion capacities of SK-Hep1 cells. Scale bar, 200 μ m. F. Sphere-forming assays showed that spheroid formation was attenuated in AGR2-deficient YY8103 cells, but enhanced in AGR2-overexpressing SK-Hep1 cells. Scale bar, 100 μ m. Bar graphs represent mean \pm SEM (n=3, *P < 0.05, **P < 0.01, and ***P < 0.001).

A

3'UTR Exons:

AGAAAAAAAAATCTCCAAGCCCTTCTGTCTGTCAGGCCTTGAGACTTGAAACCAGAAGAAGT-
GTGAGAAGACTGGCTAGTGTGGAAGCATAGTGAACACACTGATTAGGTTATGGTTTAATGTTACAACA
ACTATTTTTTAAGAAAAACAAGTTTTAGAAATTTGGTTTCAAGTGTACATGTGTGAAAAACAATATTGTAT
ACTACCATAGTGAGCCATGATTTTCTAAAAAATAAATGTTTTGGGGGTGTTCTGTTTTCTCAA
CTTGGTCTTTCACAGTGGTTCGTTTACCAAATAGGATTAACACACACAAAATGCTCAAGGAAGGGAC
AAGACAAAACCAAACTAGTTCAAATGATGAAGACCAAAGACCAAGTTATCATCTCACCACACCACAG
GTTCTCACTAGATGACTGTAAGTAGACACGAGCTTAATCAACAGAAGTATCAAGCCATGTGCTTTAGC
ATAAAGAATATTTAGAAAAACATCCCAAGAAAATCACATCACTACCTAGAGTCAACTCTGGCCAGGAA
CTCTAAGGTACACACTTTCATTTAGTAATTAATTTTAGTCAGATTTTGCCCAACCTAATGCTCTCAGG
GAAAGCCTCTGGCAAGTAGCTTTCTCCTTCAGAGGTCTAATTTAGTAGAAAGGTCATCCAAAGAACAT
CTGCACTCCTGAACACACCCTGAAGAAATCCTGGGAATTGACCTTGTAATCGATTTGTCTGTCAAGGT
CCTAAAGTACTGGAGTGAAATAAATTCAGCCAACATGTGACTAATTGGAAGAAGAGCAAAGGGTGGT
GACGTGTTGATGAGGCAGATGGAGATCAGAGGTTACTAGGGTTTAGGAAACGTGAAAGGCTGTGGCA
TCAGGGTAGGGGAGCATTCTGCCTAACAGAAATTAGAATTGTGTGTTAATGTCTTCACTCTATACTTAA
TATATGGAATTCCTCTACTGCCAGCCCCTCTGATTTCTTGCCCCCTGGACTATGGT-
GCTGTATATAATGCTTTCAGTATCTGTTGCTTGCTTGATTAACTTTTTGGATAAAACCTTTTTTGAA
CAGA

5'UTR Exons:

ACTCAGAAGCTTGGACCGCATCCTAGCCGCCGACTCACACAAGGCAGGTGGGTGAG-
GAAATCCAGAGTTGCC

CDS Exons:

ATGGAGAAAATCCAGTGTGAGCATTCTTGCTCCTTGTTGGCCCTCTCCTACACTCTGGCCA-
GAGATACCACAGTCAAACCTGGAGCCAAAAAGGACACAAAGGACTCTCGACCCAACTGCCCCAGA
CCCTCTCCAGAGGTTGGGGTGACCAACTCATCTGGACTCAGACATATGAAGAAGCTCTATATAATC
CAAGACAAGCAACAAACCTTGATGATTATTCATCACTTGGATGAGTGCCACACAGTCAAGCTTTAA
AGAAAGTGTGTTGCTGAAAATAAAGAAATCCAGAAATTGGCAGAGCAGTTGTCTCCTCAATCTGGTT
TATGAAACAACTGACAAACACCTTCTCCTGATGGCCAGTATGTCCCCAGGATTATGTTTGTGACCC
ATCTCTGACAGTTAGAGCCGATATCACTGGAAGATATTCAAATCGTCTCTATGCTTACGAACCTGCAG
ATACAGCTCTGTTGCTTGACAACATGAAGAAAGCTCTCAAGTTGCTGAAGACTGAATTGTAA

Figure S8 A. The full-length, 3'UTR, 5'UTR, and CDS sequences of AGR2 mRNA (data from UCSC).

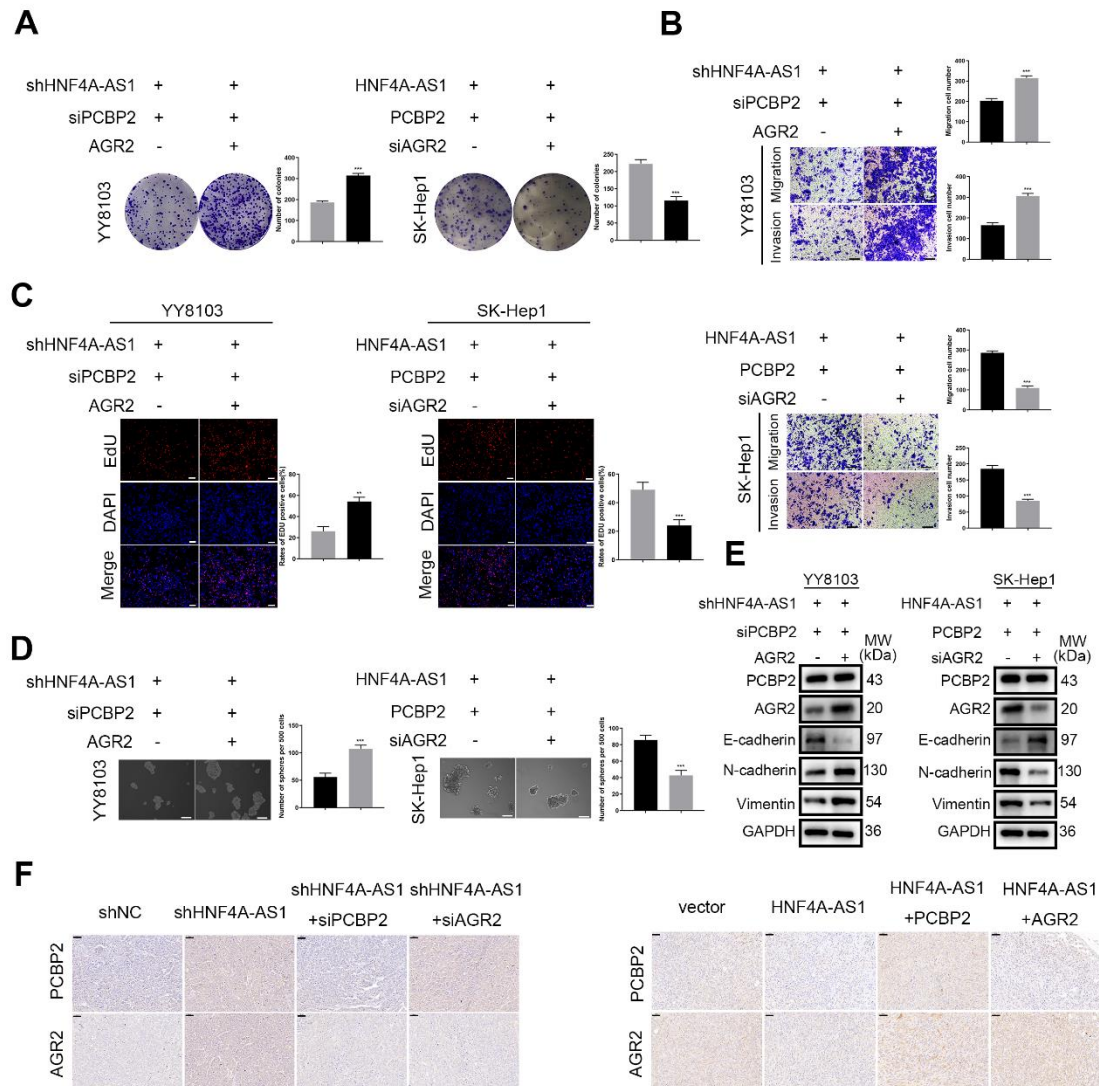


Figure. S9 HNF4A-AS1/PCBP2 regulates the progression of HCC through AGR2. A-B. Colony formation (A) and EdU assays (B) showed that the overexpression of AGR2 restored the proliferation of HNF4A-AS1-knockdown and PCBP2-knockdown YY8103 cells, while the knockdown of AGR2 reduced the proliferation of SK-Hep1-HNF4A-AS1+PCBP2 cells. Scale bar, 50 μ m. C. Transwell assays demonstrated that overexpression of AGR2 counteracted the effect of HNF4A-AS1 and PCBP2 overexpression on the metastasis of YY8103 cells. Additionally, knockdown of AGR2 restored the invasion and migration of SK-Hep1 cells with HNF4A-AS1 and PCBP2 knockdown. Scale bar, 100 μ m. D. Sphere-forming assays showed that AGR2 restored the impact of HNF4A-AS1/PCBP2 on the

stemness of HCC cells. E. EMT-associated proteins were detected by western bolt analysis, which demonstrated that AGR2 restored the inhibiting impact of HNF4A-AS1/PCBP2 on EMT in HCC cells. F. Immunohistochemistry of PCBP2 and AGR2 in subcutaneous tumors. Scale bar, 50 μ m. Bar graphs represent mean \pm SEM (n=3, *P < 0.05, **P < 0.01 and ***P < 0.001)

Supplemental table 1 Sequence for shRNA and siRNA

shHNF4A-AS1	CCAACCACTGACCAAACCTCC GACTCGTTCCACAACGCGGA
si-PCBP2	CCATGATCCATCTGTGTAGTT CCCACTAATGCCATCTTCAAA
si-AGR2	TTCTGAGTTAGCAACAAGTAA AAGCTCTATATAAATCCAAGA
si-AIP4	AAGTGCTTCTCAGAATGATGA AACCACAACACACGAATTACA

Supplemental table 2 Primers used for quantitative RT-PCR

Target	Direction	Sequence
HNF4A-AS1	Forward	5'- TCGGTGACTTGAAGACAGGC -3'
	Reverse	5'- CCAGGTGTGACACAGGTTGT -3'
PCBP2	Forward	5'- CTTTGGCTGGACCCACTAATG-3'
	Reverse	5'- CCCTGTACTCTCTCGTATTTCT-3'
AGR2	Forward	5'- AGGACTCTCGGCCCAAACCTA-3'
	Reverse	5'- GCTTGAAGTGTGTGGGCATTC-3'
EpCAM	Forward	5'-AATCGTCAATGCCAGTGTACTT-3'
	Reverse	5'-TCTCATCGCAGTCAGGATCATAA-3'
CD44	Forward	5'-TCCAACACCTCCCAGTATGACA-3'
	Reverse	5'-GGCAGGTCTGTGACTGATGTACA-3'
CD133	Forward	5'-ACTCGG CTCCCTGTTGCTGCT-3'
	Reverse	5'-GAGCCTGATATC CTGACCATTG-3'
CD90	Forward	5'-TCACCCATCCAGTACGAGTTC-3'
	Reverse	5'-GGAGCGGTATGTGTGCTCAG-3'

GAPDH	Forward	5'-GGGGCAGTTATTGCACTTGTC-3'
	Reverse	5'-AGGGGCCATCCACAGTCTTC-3'

Supplemental table 3 Antibodies used in this study.

Antibody	Company	Catalog Number
PCBP2	Proteintech	15070-1-AP
AIP4	Proteintech	20920-1-AP
AGR2	Proteintech	12275-1-AP
E-Cadherin	Proteintech	20874-1-AP
N-Cadherin	Proteintech	66219-1-Ig
Vimentin	Proteintech	10366-1-AP
Flag	Proteintech	80010-1-RR
HA	Proteintech	51064-2-AP
His	Proteintech	66005-1-Ig
Ubiquitin	Cell Signaling Technology	3936

Supplemental Table 5. CPAT prediction of HNF4A-AS1

Sequence Name	RNA size	ORF size	Ficket Score	Hexamer Score	Coding Probability	Coding Label
NR_109949.1	672	90	0.9976	0.128086415	0.039751237	no