Supplementary figure legend, figures and Table

Figure S1 Expression of ARID2 protein was reduced in high metastatic HCC cells. Western blot examined the level of ARID2 protein in low metastatic (PLC/PRF/5, HepG2, Hep3B, YY-8103 and Huh7) and high metastatic (HCCLM3, MHCC97-H and PVTT) HCC cells. GAPDH used as a loading control.

Figure S2 ARID2 suppressed the migration and invasion of HCC cells *in vitro*. (A) Migrated and invasive cells of ARID2 overexpressing MHCC97-H and PVTT cells. Scale bar, 250μm (migration), 125μm (invasion). (B) Migrated and invasive cells of ARID2 knockdown PLC/PRF/5 and YY-8103 cells. Scale bar, 250μm (migration), 125μm (invasion)

Figure S3 ARID2 inhibited metastasis of intrahepatic metastasis mouse model and primary HCC mouse models. (A), (B) Haematoxylin-eosin (HE) staining and Glypican-3 immunostaining of primary tumors and metastatic lesions in intrahepatic injection mouse model and primary HCC mouse models. Scale bar, 250μm.

Figure S4 ARID2 reduced Snail expression while increased E-cadherin expression in hepatocytes and mouse models. (A) The protein levels of EMT associated factors in *Arid2* knockout hepatocytes were investigated by

western blot. Tubulin used as a loading control. (B) mRNA levels of Snail and E-cadherin in *Arid2* knockout hepatocytes were examined by RT-qPCR. The ARID2 mRNA levels were normalized to the GAPDH mRNA levels. (C), (D) E-cadherin immunostaining in metastatic lesions of intrahepatic metastasis mouse model and primary tumors and metastatic lesions of HCC mouse models. Scale bar, 25μm. *PK*: $P53^{fl/fl}$; $LSL\text{-}Ras^{G12D}$; *AlbCre. PKA*: $P53^{fl/fl}$; $LSL\text{-}Ras^{G12D}$; $Arid2^{fl/fl}$; *AlbCre.* All * $P<0.05$, ** $P<0.01$.

Figure S5 Snail mediated the suppressive effect on migration, invasion and metastasis by ARID2 in HCC cells. (A) The protein levels of Snail and ARID2 were examined by western blot. Cell migration (B) and invasion (C) were examined by Boyden chamber and transwell assay in ARID2-overexpressing (upper panel) and knockdown cells (lower panel) with modified Snail expression. Scale bar, 100μm. (D) Intrahepatic metastasis of ARID2-overexpressing (left, n=6) and knockdown cells (right, n=7) with modified Snail expression was examined, and number of superficial foci in injection lobe and non-injection lobes were counted. Scale bar, 250μm. (E) Fluorescence of metastases generated by ARID2-overexpressing (upper, n=5) and knockdown cells (lower, n=5) with modified Snail expression in distant seeding mouse model was measured. Data were analyzed using Student's *t*-test. All * $P<0.05$, ** $P<0.01$, *** $P<0.001$, ns: not significant.

Figure S6 Inhibition of DNMT1 restored the migration and invasion of HCC cells overexpressing ARID2. (A), (B) The migration and invasion of ARID2-overexpressing MHCC97-H and PVTT cells treated with shRNA

targeted DNMT1 or DNMT1 inhibitor 5-AZA (5μM) and 6-TG (3μM) for 48 hours. Scale bar, 100μm (migration); scale bar, 500μm (invasion).

Figure S1

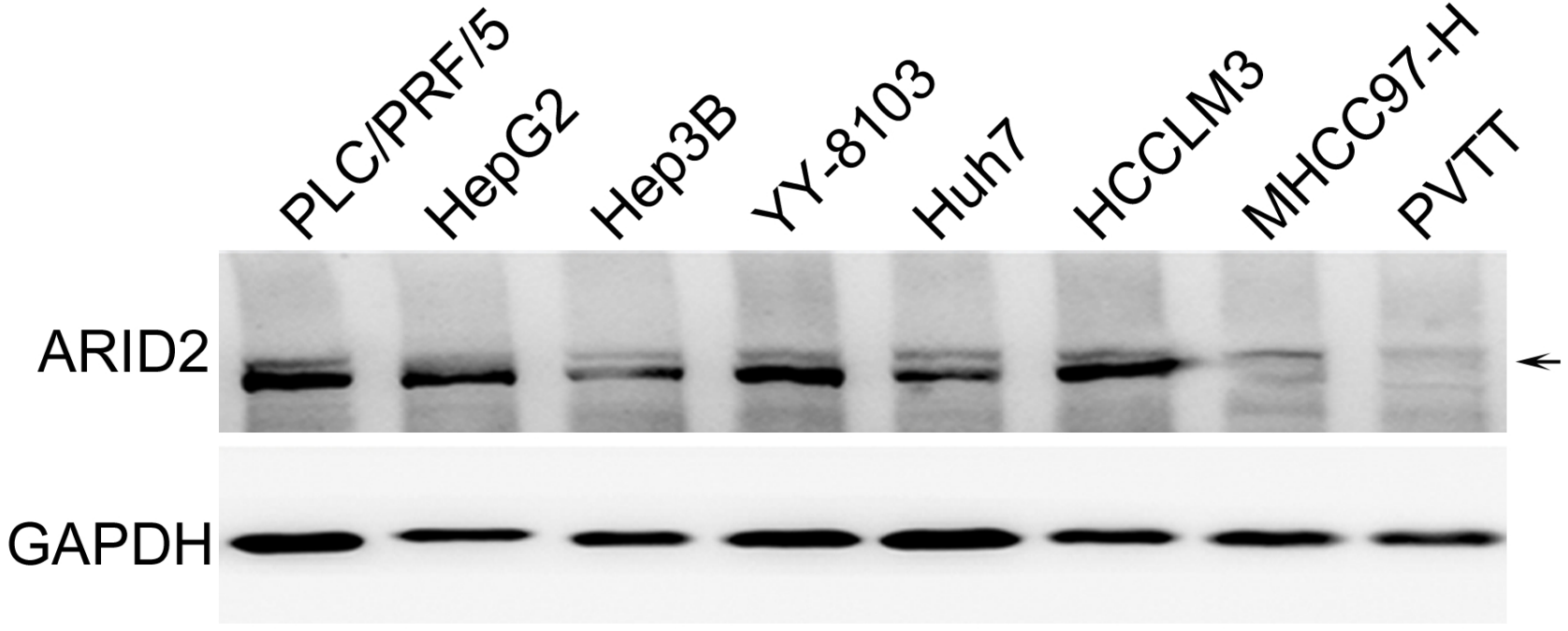
PLC/PRF/5
HepG2
Hep3B
YY-8103
Huh7
HCCLM3
MHCC97-H
PVTT
ARID2
GAPDH

Figure S2

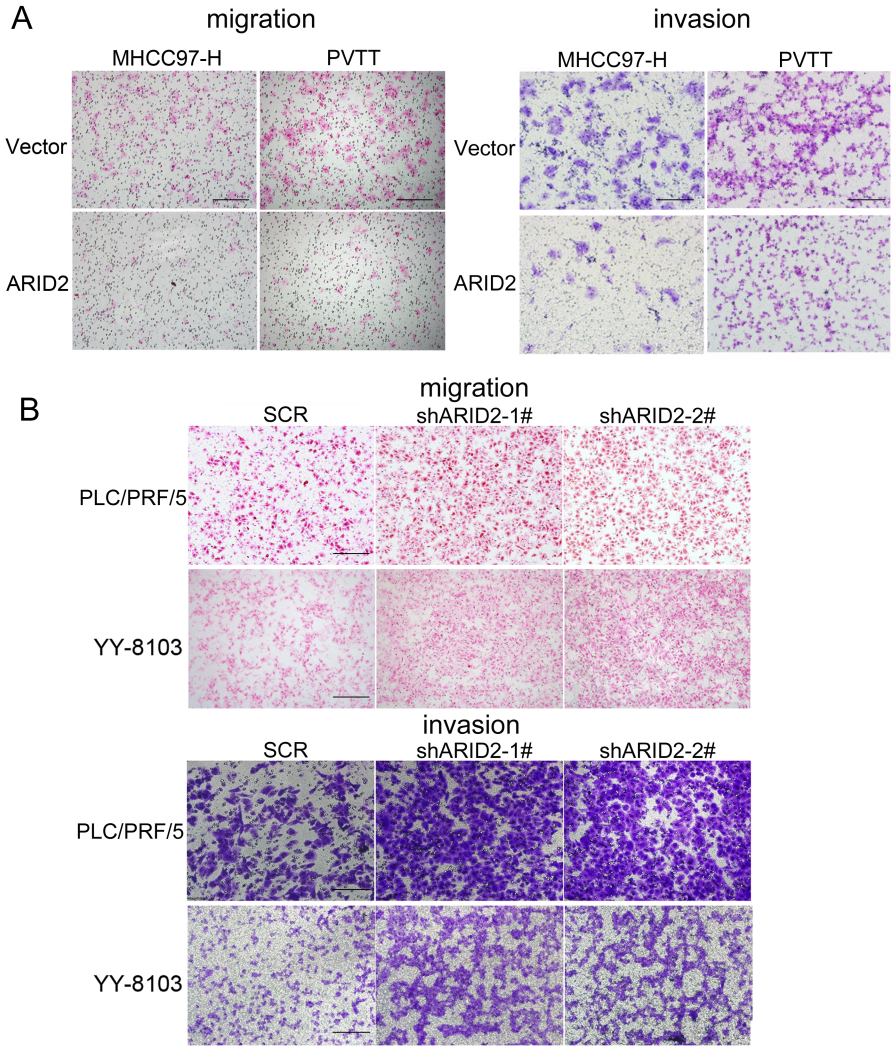
A
migration
invasion
MHCC97-H
PVTT
MHCC97-H
PVTT
Vector
ARID2
Vector
ARID2
B
migration
SCR
shARID2-1#
shARID2-2#
PLC/PRF/5
YY-8103
invasion
SCR
shARID2-1#
shARID2-2#
PLC/PRF/5
YY-8103

Figure S3

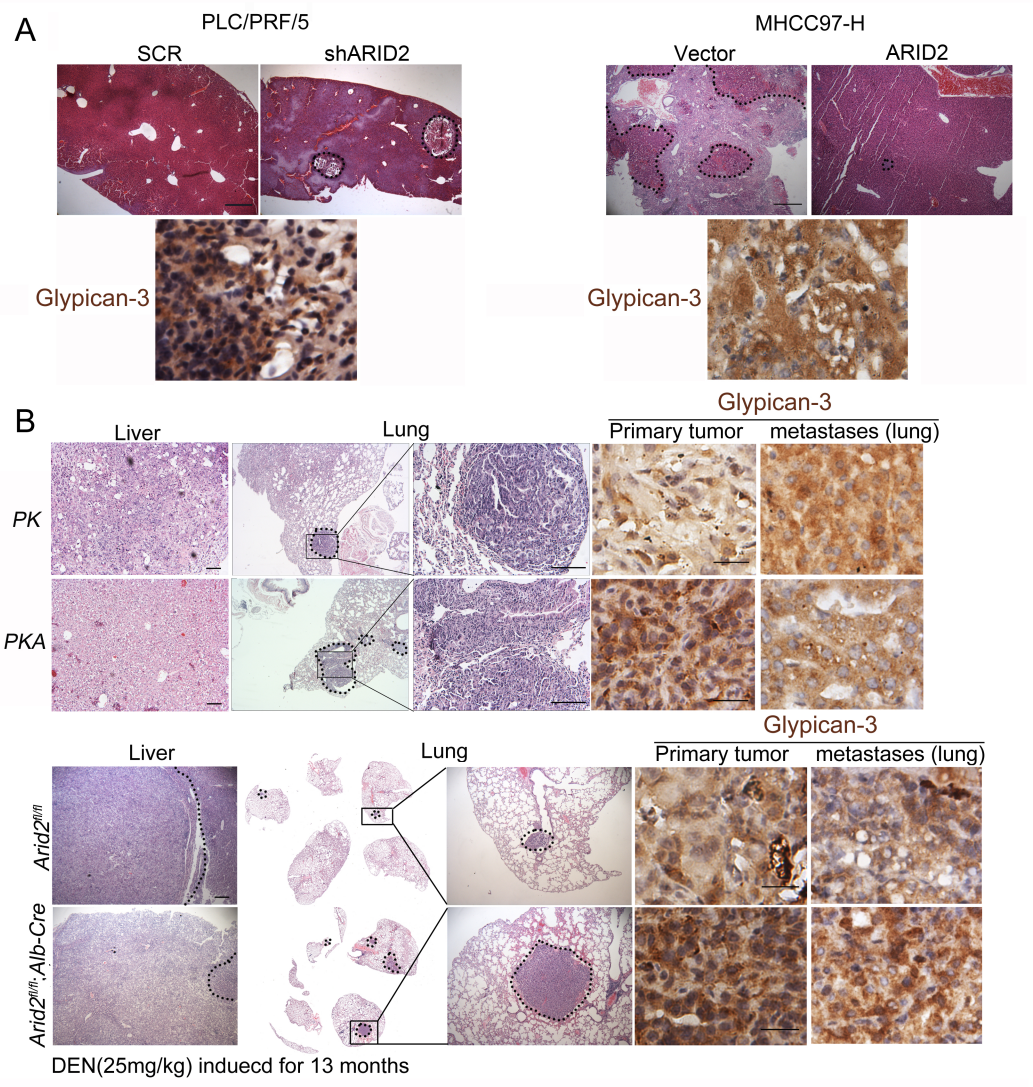
A
PLC/PRF/5
SCR
shARID2
Glypican-3
MHCC97-H
Vector
ARID2
Glypican-3
B
Liver
Lung
Glypican-3
Primary tumor
metastases (lung)
PK
PKA
Liver
Lung
Glypican-3
Primary tumor
metastases (lung)
Arid2flox/flox
Arid2flox/flox;Alb-Cre
DEN(25mg/kg) induecd for 13 months

Figure S4

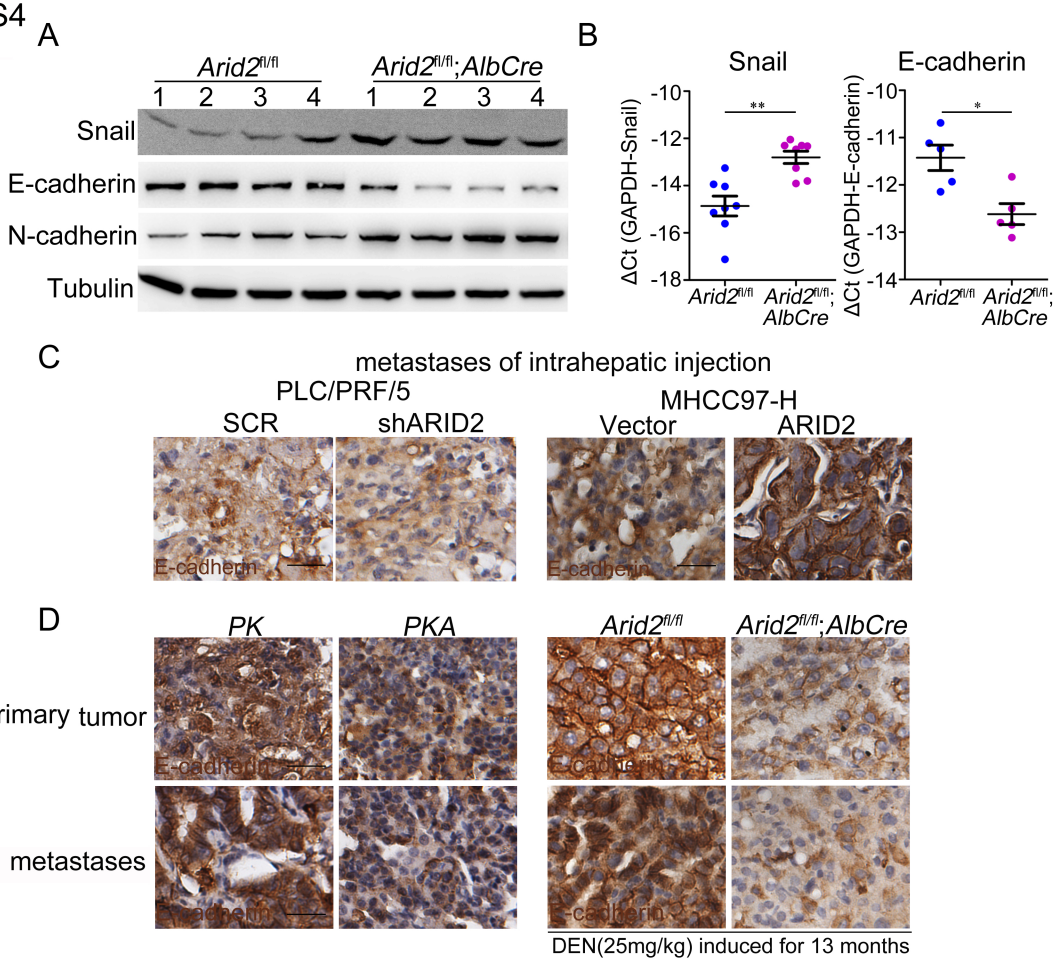
A
Arid2fl/fl
Arid2fl/fl;AlbCre
1 2 3 4 1 2 3 4
Snail
E-cadherin
N-cadherin
Tubulin
B
Snail
E-cadherin
ΔCt (GAPDH-Snail)
ΔCt (GAPDH-E-cadherin)
-10
-12
-14
-16
-18
-11
-13
**
*
Arid2fl/fl
Arid2fl/fl; AlbCre
C
metastases of intrahepatic injection
PLC/PRF/5
SCR
shARID2
MHCC97-H
Vector
ARID2
E-cadherin
D
PK
PKA
Arid2fl/fl
Arid2fl/fl;AlbCre
primary tumor
metastases
DEN(25mg/kg) induced for 13 months

Figure S5

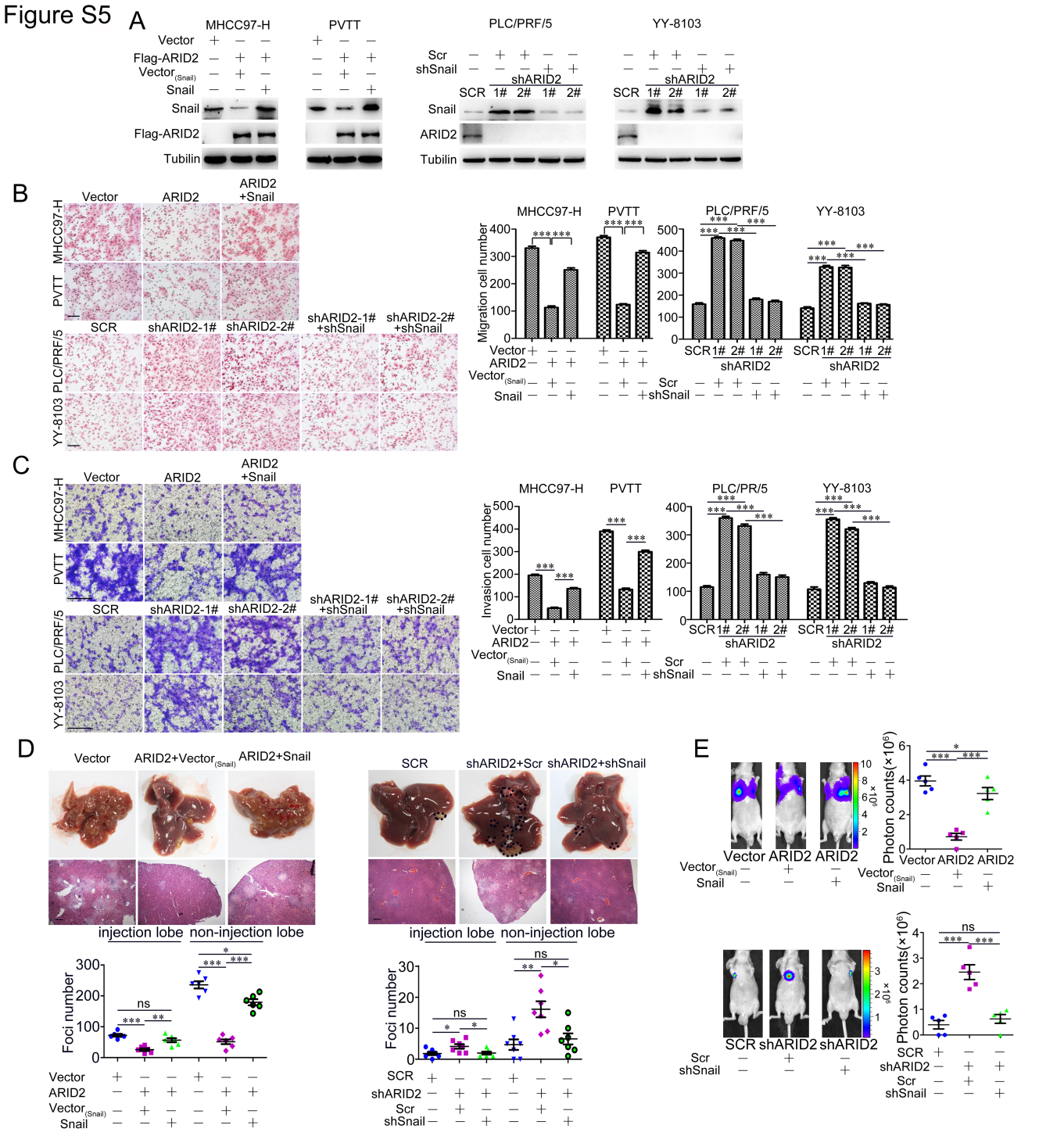
A
MHCC97-H
PVTT
PLC/PRF/5
YY-8103
Vector
Flag-ARID2
Vector(Snail)
Snail
Tubilin
Scr
shSnail
shARID2
SCR
1#
2#
ARID2
B
Vector
ARID2
ARID2 +Snail
MHCC97-H
PVTT
PLC/PRF/5
YY-8103
SCR
shARID2-1#
shARID2-2#
shARID2-1# +shSnail
shARID2-2# +shSnail
Migration cell number
C
Invasion cell number
D
Vector
ARID2+Vector(Snail)
ARID2+Snail
SCR
shARID2+Scr
shARID2+shSnail
injection lobe
non-injection lobe
Foci number
E
Photon counts(×10^6)
ns

Figure S6

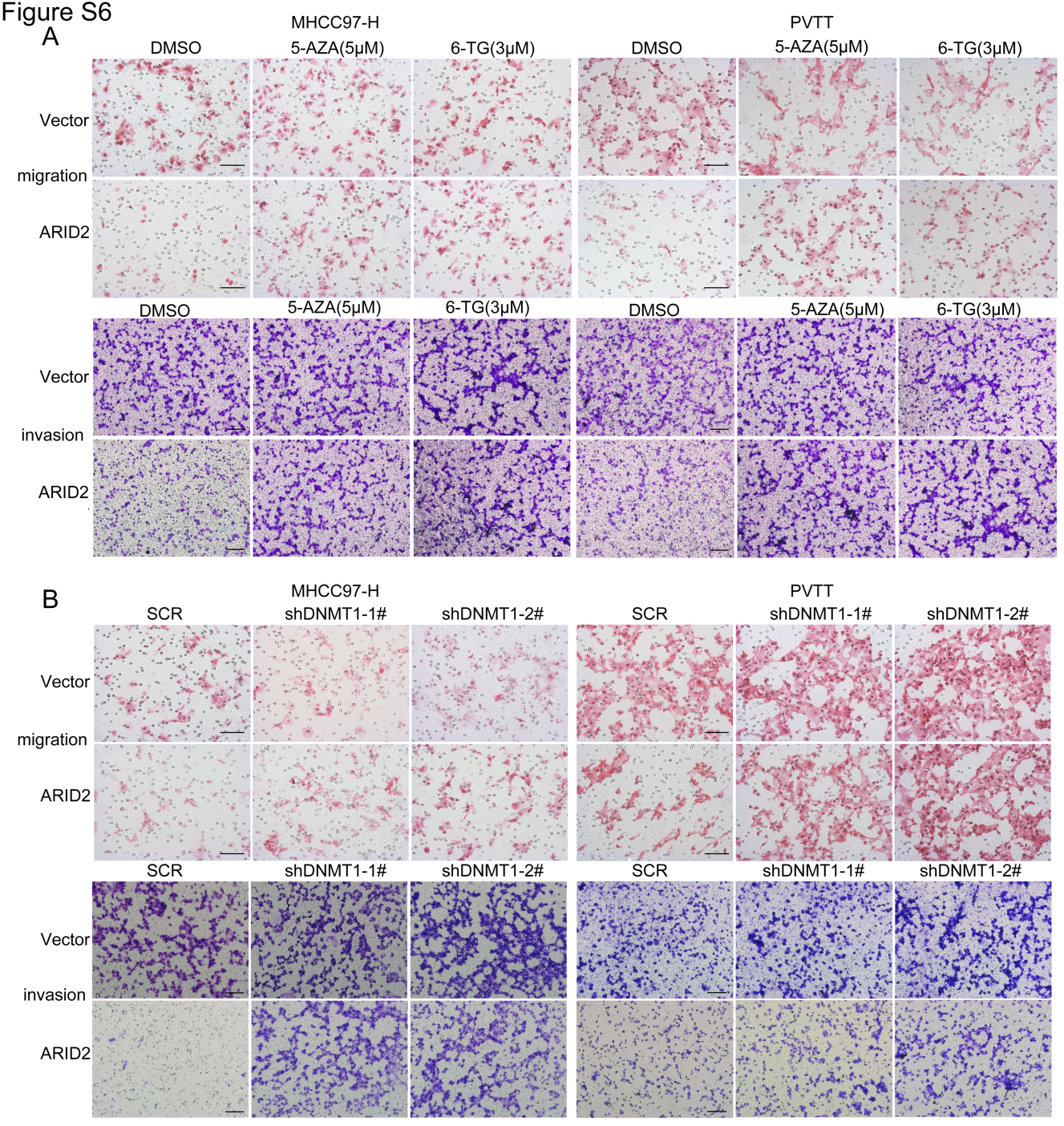
A
MHCC97-H
PVTT
DMSO
5-AZA(5μM)
6-TG(3μM)
DMSO
5-AZA(5μM)
6-TG(3μM)
Vector
migration
ARID2
DMSO
5-AZA(5μM)
6-TG(3μM)
DMSO
5-AZA(5μM)
6-TG(3μM)
Vector
invasion
ARID2
B
MHCC97-H
PVTT
SCR
shDNMT1-1#
shDNMT1-2#
SCR
shDNMT1-1#
shDNMT1-2#
Vector
migration
ARID2
SCR
shDNMT1-1#
shDNMT1-2#
SCR
shDNMT1-1#
shDNMT1-2#
Vector
invasion
ARID2

Table S1 Correlation between the ARID2 expression and the clinicopathologic features of HCC.

Characteristic	Total	ARID2(n%)		χ2	*P* value
		High	Low		
	287	n=84	n=203		
Gender				0.570	0.450
Male	257	50	207		
Female	30	7	23		
Age(years)				0.244	0.621
>=50	120	37	83		
<50	167	47	120		
Tumor size				11.714	0.003*
>=12	88	19	69		
>12>=7	125	32	93		
<7	74	33	41		
Pathological grade				4.109	0.043*
Well	220	71	149		
Poor	67	13	54		
Lymph node metastasis				1.941	0.164
Yes	15	2	13		
No	272	82	190		
TNM stage				0.706	0.401
Ⅰ-Ⅱ	37	13	24		
Ⅲ-Ⅳ	250	71	179		
Tumor number					
>=2	29	8	21	0.044	0.834
<=1	258	76	182		
Organ metastasis				4.536	0.033*
Yes	76	15	61		
No	211	69	142		

Statistical analyses were carried out using Chi-square χ2 test

* represented $p<0.05$ was considered significant

Table S2 Primers of gene clone.

Primers	Sequence
ARID2 clone	F: 5’-ATAAGAATGCGGCCGCATGGCAAACTCGACGGGGAAG-3’ R: 5’-CGACGCGTGCTGCAGCATTTCTGAGTCTTTTTCT-3’
ARID2 mutant	F: 5’-ACCTGCAACTGTGAGTCAGGGAAAT-3’ R: 5’-GTTCCATTGGAGATATCTCACCATT-3’
Snail clone	F: 5‘-CGGGATCCATGCCGCGCTCTTTCCTCGTC-3’ R: 5'-CCATCGATTCAGCGGGGACATCCTGAGCA-3'
ARID2 (1-303)	F:5’-ATAAGAATGCGGCCGCCATGGCAAACTCGACGGGGAAG-3’ R: 5'-GAAGATCTTCACTCGTACTTTTCTAGGTAACG-3’
ARID2 (1-1746)	F:5'-ATAAGAATGCGGCCGCCATGGCAAACTCGACGGGGAAG-3’ R: 5’-GAAGATCTTCATCTCTTCACTGTATGATTTG-3’
ARID2 (1-3384)	F:5’-ATAAGAATGCGGCCGCCATGGCAAACTCGACGGGGAAG-3’ R: 5’-GAAGATCTTCACTGAACATTTTGCTGCCCAAC-3’
ARID2(wt)	F:5’-ATAAGAATGCGGCCGCCATGGCAAACTCGACGGGGAAG-3’ R: 5’-GAAGATCTTCACTGCAGCATTTCTGAGTCTTTT-3’
Snail promter (-2340-+146)	F: 5’-TATAGGTACCTGGGCTGGACAACAGTTCTTTAC-3’
Snail promter (-2340-+146)	R: 5’-ATATAAGCTTTCGCTGTAGTTAGGCTTCC-3’

Table S3 Primers of shRNA.

Primers	Sequence
Scramble	F: 5’-CCGGCCTAAGGTTAAGTCGCCCTCGCTCGAGC GAGGGCGACTTAACCTTAGG-3’ R: 5’-AATTCCCTAAGGTTAAGTCGCCCTCGCTCGAGC
ARID2 shRNA1	F: 5’-CCGGCCGACTAACAGCTGCCTTAATCTCGAGATT AAGGCAGCTGTTAGTCGGTTTTTG-3’ R: 5'-AATTCAAAAACCGACTAACAGCTGCCTTAATCTC GAGATTAAGGCAGCTGTTAGTCGG-3’
ARID2 shRNA2	F: 5’-CCGGGCAGGATAAGCACTGTTCAAACTCGAGTT AAGGCAGCTGTTAGTCGGTTTTTG-3’ R: 5’-AATTCAAAAAGCAGGATAAGCACTGTTCAAACTC GAGTTTGAACAGTGCTTATCCTGC-3’
DNMT1 shRNA1	F: 5’-CCGGCGAGAAGAATATCGAACTCTTCTCGAGAA GAGTTCGATATTCTTCTCGTTTTTG-3’ R: 5’-AATTCAAAAACGAGAAGAATATCGAACTCTTCTC GAGAAGAGTTCGATATTCTTCTCG-3’
DNMT1 shRNA2	F: 5’-CCGGGCCCAATGAGACTGACATCAACTCGAGTT GATGTCAGTCTCATTGGGCTTTTTG-3’ R: 5’-AATTCAAAAAGCCCAATGAGACTGACATCAACTC GAGTTGATGTCAGTCTCATTGGGC-3’
Snail shRNA1	5'-CCGGCCAATCGGAAGCCTAACTACACTCGAGTGTA GTTAGGCTTCCGATTGGTTTTTG-3' 5'-AATTCAAAAACCAATCGGAAGCCTAACTACACTCGA GTGTAGTTAGGCTTCCGATTGG-3'
Snail shRNA2	5'-CCGGCCACTCAGATGTCAAGAAGTACTCGAGTACT TCTTGACATCTGAGTGGTTTTTG-3' 5'-AATTCAAAAACCACTCAGATGTCAAGAAGTACTCGA GTACTTCTTGACATCTGAGTGG-3'

Table S4 Primers of RT-qPCR.

Primers	Sequence
human-GAPDH	F: 5’- ATGACCCCTTCATTGACCTCA-3’
	R: 5’- GAGATGATGACCCTTTTGGCT-3’
human-ARID2	F: 5’-CAGTGTGTCGGATTATCTGCG-3’
	R: 5’-GCATGACGTGCTTGCTTTCATT-3’
human-E-caherin	‘F: 5’-ATTTTTCCCTCGACACCCGAT-3’
	R: 5’-TCCCAGGCGTAGACCAAGA-3’
human-N-caherin	F: 5’-TGCGGTACAGTGTAACTGGG-3’
	R: 5’-GAAACCGGGCTATCTGCTCG-3’
human-Snail	F: 5’-CGCCTGACTGAGCAACTGG-3’
	R: 5’-GGAAGCTGGCGGTACTTGT-3’
human-β-catenin	F: 5’-CATCTACACAGTTTGATGCTGCT-3’
	R: 5’-GCAGTTTTGTCAGTTCAGGGA-3’
human-DNMT1	F: 5’-AGGCGGCTCAAAGATTTGGAA-3’
	R: 5’-GCAGAAATTCGTGCAAGAGATTC-3’
mouse-GAPDH	F: 5’-AGGTCGGTGTGAACGGATTTG-3’
	R: 5’-TGTAGACCATGTAGTTGAGGTCA-3’
mouse-Snail	F: 5’-CACACGCTGCCTTGTGTCT-3’
	R: 5’-GGTCAGCAAAAGCACGGTT-3’
mouse-E-caherin	F: 5’-CAGTTCCGAGGTCTACACCTT-3’
	R: 5’-TGAATCGGGAGTCTTCCGAAAA-3’
mouse-N-caherin	F: 5’-AGCGCAGTCTTACCGAAGG-3’
	R: 5’-TCGCTGCTTTCATACTGAACTTT-3’

Table S5 Chip primers of Snail promoter and primers for quantifying the methylation level of Snail promoter promoter.

Primers	Sequence
GAPDH	F: 5’-CGTAGCTCAGGCCTCAAGAC-3’
(chr12:6643561-6643672)	R: 5’-GCTGCGGGCTCAATTTATAG-3’
methylation primer1	F: 5’-CCAGGTACAGTGCCCCAC-3’
(-739～-583bp)	R: 5’-AGGAAGCGAGGAAAGGGA-3’
methylation primer2	F: 5’-TTGCCACTTCTTCCCTCG-3’
(-979～-882bp)	R: 5’-ACCGTTAAGAGGCGGGTC-3’
methylation primer3	F: 5’-TCAGAAGCGCTCAGACCA-3’
(-438～-161bp)	R: 5’-CCCCTTTGTCACCTCCG-3’
chip primer1	F: 5’-AAAGGGGCGTGGCAGATA-3’
(-168～-31bp)	R: 5’-GCAGCAGTAGCGCAGAAGA-3’
chip primer2	F: 5’-CCCAGGTACAGTGCCCCACA-3’
(-740～-615bp)	R: 5’-CTCGGCGGCTTGAAATG-3’
chip primer3	F: 5’-CAGGTGACCCGCCTCTTA-3’
(-905～-805bp)	R: 5’-GGGAGACAGACGAAGTAAACAG-3’
chip primer4	F: 5’-GCCCCTATGGAGCCGTGTT-3’
(-1270～-1135bp)	R: 5’-GACCTGGTTAGAGTTTCGTTG-3’
chip primer5	F: 5’-CCAATCGGAAGCCTAACTACA-3’
(37～211bp)	R: 5’-CCGCAATGGTCCACAAAAC-3’

Methods and materials

Cell lines and tumor samples

PVTT cells was established by our lab, the details of establishing PVTT cell line and clinical information about the patient provided the tumor tissue has been described previously. HEK293T and other human HCC cells were purchase from Cell Bank of Type Culture Collection of Chinese Academy of Sciences, Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Dulbecco's Modified Eagle's Medium (DMEM, Gibco) Supplemented with 1% antibiotics and 10% fetal bovine serum (FBS) were used to culture the cells, the culture incubator was sterile at 37℃ with 5% CO_2.

After obtaining written informed consent, all HCC and paired adjacent tissues were collected from Eastern Hepatobiliary Surgery Hospital, Second Military Medical University (Shanghai, China) between 2013 and 2015. To evaluate the expression of ARID2 and Snail, we examined the tissues array with different cohort of HCC patients and analyzed the correlation between ARID2 and clinical features, Snail expression. These experiments were approved by the Ethical Committee of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China) following declaration of Helsinki ethical guidelines.

Plasmids and transfection

ARID2 coding sequence was inserted into

pHAGE-full-EF1a-MCS-IZsGreen lentiviral vector and cells transfected with ARID2 gene were screened using flow cytometry by green fluorescence. Snail gene was cloned into pHAGE-full-EF1a-MCS-Puro lentiviral vector and positive cells were screened by puromycin (3μg/mL) for 4 days. Truncations of ARID2 were constructed using 3×Flag-CMV-10 vector and DNMT1 was inserted into Tag2B-myc vector. shRNAs, targeted ARID2 and DNMT1, were designed using software provided by Qiagen (Valencia, CA, USA) and inserted into pLKO.1-TRC vector. The lentivirus was packaged in HEK293T cells and harvested under the condition 4℃ 20000 RPM for 2 hours. After dissolving the lentiviral particles with DMEM medium, Cells (2×10^5) in 6-well plate were incubated with lentiviral particles for 8 hours. Positive cells were selected by puromycin (4μg/mL) for 4 days. The resistant cells were pooled and examined the expression of target genes. The sequences of clone gene and shRNA were listed in **Table S2** and **Table S3**, respectively.

HCC mouse model

LSL-Ras^{G12D}, *P53$^{fl/fl}$* and *Alb-Cre* mice were obtained from Jackson Laboratory and *Arid2$^{fl/fl}$* mice was provided form Bin Zhou' s lab (Institute for Nutritional Sciences, Chinese Academy of Sciences, Shanghai, China). *Alb-Cre; Arid2$^{fl/fl}$* mice was generated by crossing *Alb-Cre* mice with *Arid2$^{fl/fl}$* mice and *Alb-Cre; Arid2$^{fl/fl}$*; *LSL-Ras^{G12D}*; *P53$^{fl/fl}$* mice was bred by crossing *Alb-Cre* mice with *Arid2$^{fl/fl}$*; *LSL-Ras^{G12D}* and *P53$^{fl/fl}$* mice. Metastases were

counted under microscope. All mice were male in C57BL/6 background and all animal experiments were performed under the approval of the Institutional Animal Care and Use Committee (IACUC).

Real-time quantitative PCR (qPCR)

Total RNA was extracted from cells and clinical HCC tissues with TRIzoL (Invitrogen) following the manufacturer's instructions and 2μg RNA was reversed transcribed into cDNA using the reversing transcriptase kit (Promega). mRNA level was detect by thermo scientific detection system (PIKOREAL96) using 2×SYBR Green kit (Takara). The conditions of PCR were: 40 cycles of 95°C for 20 s, 60°C for 30 s, and 72°C for 30 s, and one cycle of 72°C for 10 minutes. The sequences of primers were provided in **Table S4**.

Western blot

After washing by phosphate buffer saline (1.37M NaCl, 27mM KCl, 100mM Na_2HPO_4, 20mM KH_2PO_4, pH=7.4) twice, cell was lyzed with RIPA solution (50mM Tris-HCl (pH=7.4), 150nM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% Nonidet P-40) supplemented with protease and phosphatase inhibitors (Sigma) to extract protein and protein was quantified by Bradford reagent (Bio-Rad). Before incubated with antibody, proteins were separated by SDS-PAGE and transferred onto PVDF membranes. Antibody against ARID2 (1:2000, Abiocade), DNMT1 (1:1000, Abcam), GAPDH (1:10000, Sigma), Flag

(Sigma, 1:4000), GST (1:1000, ProteinTech), Tubulin (1:1000, Santa Cruz Biotechnology), Twist (1:1000, Abcam), ZEB1, Snail, E-cadherin, N-cadherin, β-catenin and Slug (1:1000, Cell Signaling Technology) were used. Horseradish peroxidase-conjugated secondary antibodies (1:2,000, Cell Signaling Technology) were purchased from Cell Signaling Technology.

Immunohistochemistry (IHC)

Tissue sections paraffin-embedded were deparaffinized, rehydrated, subjected to antigen retrieval and blocked the endogenous peroxidases. After washing the section with 0.01M PBS (150 mmol/L NaCl, 8 mmol/L Na_2HPO_4 and 2 mmol/L NaH_2PO_4) for three times, sections were blocked by 0.01 mol/L PBS supplemented with 5% normal goat serum and 0.3% Triton X-100. Next, Sections were incubated with primary antibody against ARID2 (1:100, Santa Cruz Biotechnology), Snail (1:200, Abcam) and E-cadhein (1:400, Cell Signaling Technology) overnight at 4℃. Washed three times with 0.01M PBS, sections were incubated with secondary antibody (1:500) for 2 hours. After washed once with 0.01 mol/L PBS and 0.05M Tris-HCl (pH=7.6) solution two times, sections were visualized with 0.03% 3,3′-diaminobenzidine in 0.05M Tris-HCl (pH 7.6) and counter stained with haematoxylin. The extent and staining intensity of protein were scored automatically by Vectra 2 system (Perkin-Elmer, USA). Outcome of staining was using H-score, defined by the equation: H-score=100ΣPi*i as previously described. The staining intensity (i)

of the decorated tumour cells was graded from “0 to 3”, and Pi is the percentage of the stained tumour cells with various intensities.

Boyden chamber assay

3×10^5 cells were loaded into upper chamber of one Boyden chamber in 200μL medium with 1% FBS, and 250 μL medium with 10% FBS was placed into the bottom of chamber. After seven hours, migrated cells was stained with hematoxylin and eosin. Randomly selected four fields and counted the cells, experiments were repeated three times and the data are presented as the means ± SD.

Transwell assay

Invasion of HCC cells was examined by transwell with polyethylene terephthalate membranes (24-well inserts, 8.0 μm, Corning). 150 μL cell suspension contained 2×10^5 cells was loaded into upper well coated with Matrigel (BD Biosciences). Next, 500 μL DMEM medium with 10%FBS was placed into the bottom of the well to induce invasion. 72 hours later, invasive cells were staining with with 0.1% crystal violet. Randomly selected four fields and counted the cells, experiments were repeated three times and the data are presented as the means ± SD.

Immunoprecipitation

Tris-buffered saline (50 mmol/L Tris, 150 mmol/L NaCl, 1% Nonidet P-40, 1 mmol/L EDTA, 1 mmol/L Na_3VO_4, 10 mmol/L NaF, 2.5 mg/mL aprotinin, 10 mmol/L leupeptin, 1 mmol/L β-glycerophosphate, 4-(2-aminoethyl), 10 mmol/L benzenesulfonyl fluoride hydrochloride and 10 mmol/L iodoacetate , pH 7.4) was used to lysis cells and cell lysates were incubated with primary antibody against DNMT1, Myc, Flag and ARID2 at 4℃ overnight. Next day, Protein A or G beads was added and incubated for 4-6 hours. Beads were centrifuged at 4℃ at 500g for 2min and washed with Tris-buffered saline for three times. Beads was eluted with 2×loading buffer and proteins were examined by western blotting.

Luciferase reporter assay

HEK293T were placed in 24-well plate, 100ng ARID2 expression vector or empty vector, 50ng reporter plasimd and 20ng renilla luciferase were co-transfected into cells for 48 hours. However, only co-transfected the 50ng reporter plasimd and 20ng Renilla luciferase into stably-transfected HCC cell lines and its control cells for 24 hours. The experiments were performed in triplicate. Then, cells were lysed by passive lysis buffer (Promega, Madison, WI) and reporter activities were investigated by the Dual-Luciferase Reporter Assay System (Promega).

Intrahepatic metastasis model

Five-week-old nude mice was anesthetized. Then, the left lobe of liver was placed outside the body through a subcostal incision. 50 μL DMEM medium contained 5×10^5 or 1×10^5 cells was injected into the left lobe. After the wound in left lobe was haemostatic with sterile cotton tip applicator, the left lobe was back into abdominal cavity and the incision was sutured. The mice were bred freely to access to water and food. 8 or 12 weeks later, mice were euthanized. Metastases on surface of injection lobe and non-injection lobes were counted under microscope. All experiments were approved by the ethic commitment of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China).

***In vivo* metastatic assay**

To investigated effects of ARID2 on distant-seeding of HCC cells, stably-transfected HCC cells and its control cells were labelled with luciferase and 200 μL of PBS contained 1×10^6 cells was vaccinated into five-week-old nude mice by tail-vein injection. Distant-seeding lesions were monitored every week via staining with D-luciferin (150 mg/kg, Xenogen, Alameda, CA) using an in vivo imaging system (Xenogen). After injecting with D-luciferin for 2 minutes, mice were placed into the light-tight chamber and monitored by CCD camera system. Fluorescence signals from metastases were quantified using Living Image software. This experiment was approved by the ethic commitment of Shanghai Institutes for Biological Sciences, Chinese Academy

of Sciences (Shanghai, China).

Immunofluorescence

Cells plated on slides were washed three times with PBS buffer and fixed with methanol for 15 minutes at 37℃. Cells was blocked with 5% bovine serum albumin for 1hour at 37℃, and incubated with a primary antibody (DNMT1, 1:200, Abcam; ARID2, 1:200, Abiocade) overnight at 4℃. After three washes with PBS, the cells were incubated with a secondary antibody (Alexa Fluor 488-conjugated donkey anti-rabbit IgG, 1:1000; Alexa Fluor 555-conjugated donkey anti-mouse IgG, 1:1000) for 1.5 hour at room temperature and washed three times with PBS. The nuclei were stained with Hoechst dye. Fluorescence was monitored using an inverted confocal laser scanning microscope (Carl Zeiss, Thornwood, NY).

GST pull-down assay

GST-DNMT1, GST and Flag-ARID2 proteins were purified. Then, 5 μg of GST or GST-DNMT1 proteins were incubated 10 μg Flag-ARID2 protein overnight, followed by the addition of glutathione-Sepharose 4B beads for 4 hours. After centrifugation, beads were washed with Tris-buffered saline (details provided in immunoprecipitation section) for three times and eluted with 2×SDS loading buffer. Protein were examined by SDS-PAGE and western blotting.

Chromatin immunoprecipitation (ChIP) and Re-ChIP

Cell was treated with formaldehyde to cross-link the protein and DNA at 37℃ for 10 minutes and resuspended with 500 μL lysis buffer (50 mM Tris (pH=8.1), 10 mM EDTA, 1 mM PMSF and 1% SDS). The genome was fragmented by sonication under the following conditions: sonicated for 5 seconds under 300 watt of power at 4℃ and suspended for 10 seconds, repeating above steps for six times. After centrifugation, 5 μL of the supernatants were used as inputs, and the remainder diluted 4-fold in IP buffer (20 mM Tris-HCl (pH=8.1), 2 mM EDTA, 100 mM NaCl, and 0.5%Triton X-100). This diluted was pre-clear by the herring sperm DNA/Protein G-Sepharose slurry. Supernatant mixed with herring sperm DNA and Protein G-Sepharose beads incubated with ChIP grade antibody or control IgG for 4 hours. Precipitates were washed with Buffer I (2 mM EDTA, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 20 mM Tris-HCl (pH=8.0)) for three times and Buffer II (10 mM Tris-HCl (pH=8.0), 1 mM EDTA, 1% Deoxycholate, 1% NP-40, 0.25 M LiCl) once. Precipitated chromatin complexes were removed from beads by incubating with 150 μL dilution buffer (1.1M $NaHCO_3$ and 1% SDS). In Re-ChIP experiments, precipitated chromatin complexes were eluted by incubation in 25 μL 10 mM DTT for 30 min at 37℃. After centrifugation, the supernatant was diluted 20 times with Re-ChIP buffer (20 mM Tris-HCl, 1% Triton X-100, 150 mM NaCl,2 mM EDTA, pH=8.1) and subjected to the ChIP procedure again. Crosslinking was reversed by incubation at 65 for 4 hours

and DNA was purified with quick columns (Qiagen, USA). Five paired-primers were employed to assess the enrichment of ARID2 at Snail promoter and sequence of primers were provided in **Table S5**.

Methylated DNA Immunoprecipitation (MeDIP)-PCR

MeDIP was performed to evaluate the methylation level of Snail promoter using EpiQuik^{TM} methylated DNA immunoprecipitation Kit (EPIGENTEK, USA). Experiment was followed the instructions provided by the manufacturer. Cells were incubated with lysis buffer and lysates were transferred into a vial. Meanwhile, strips were coated by antibody against 5-methylcytosine and control IgG. After vortex vigorously for 10 seconds, genome was fragmented by sonication under following conditions: sonicated for 5 seconds under 300 watt of power at 4℃ and suspended for 10 seconds, repeating above steps for eight times. Supernatants diluted 1-fold in dilution buffer and removed 5 μL diluted supernatant as input. 100 μL diluted supernatant was transfer into wells on strip coated with antibody and incubated for 90 minutes. Then, after washing by washing buffer for six times and 1×TE buffer once, wells and input were incubated with 40 μL buffer mixed with protease K for 15 minutes in a 65℃ water bath. 40μL elution buffer was added into wells and input at 65℃ for 45 minutes. DNA was purified with quick columns. Purified DNA was used as the template and methylation level of Snail promoter was quantified by qPCR. The sequences of primers were provided in **Table S5**.

Statistical analysis

The correlations between the clinico-pathological features and ARID2 staining scores were analysed using the chi-square ($\chi 2$) test. Survival curves were plotted by the Kaplan-Meier method and analysed by the log-rank test. Statistical analyses were performed by GraphPad Prism 5 and SPSS17.0 software. The results are representative of at least three independent experiments performed in triplicate and are expressed as the means ± SD. The data were analysed using Student's *t*-test.