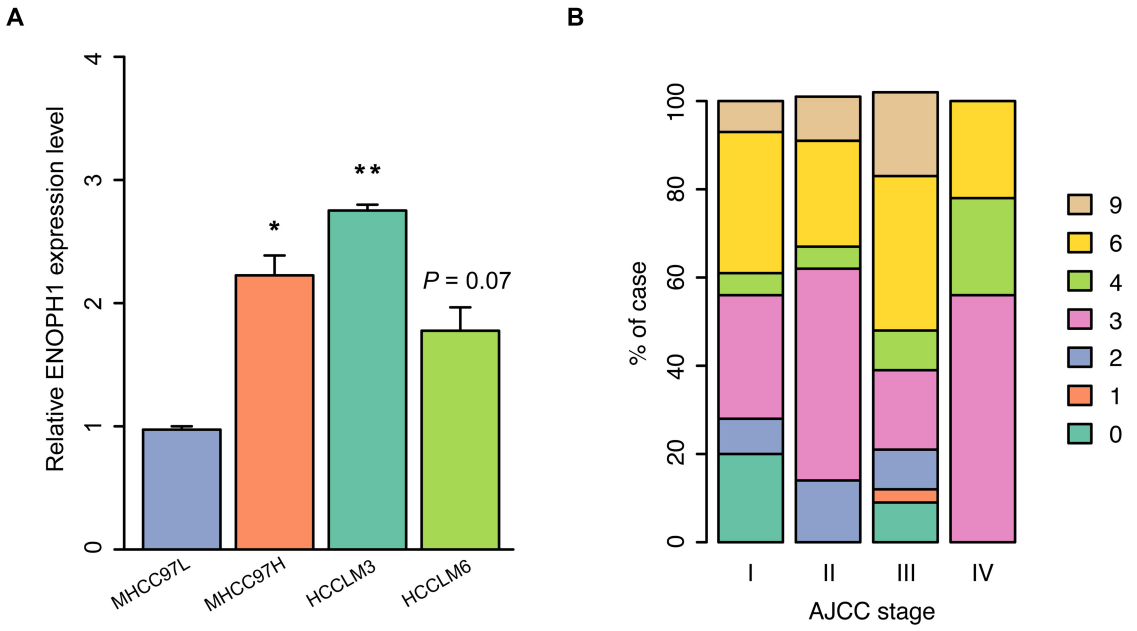


**Fig. S1**. **ENOPH1 expression correlating with HCC metastasis. a** The expression levels of ENOPH1 by qPCR using MHCC97L, MHCC97H, HCCLM3, and HCCLM6 cells. **b** Percentage chart showing the constituent ratio of cases with ENOPH1 staining scores in HCC tissues and para-tumor tissues. Data represent the means ± SEM (*:$P$ <0.05, **:$P$ <0.01, ***:$P$ <0.001, by Student's $t$-test).

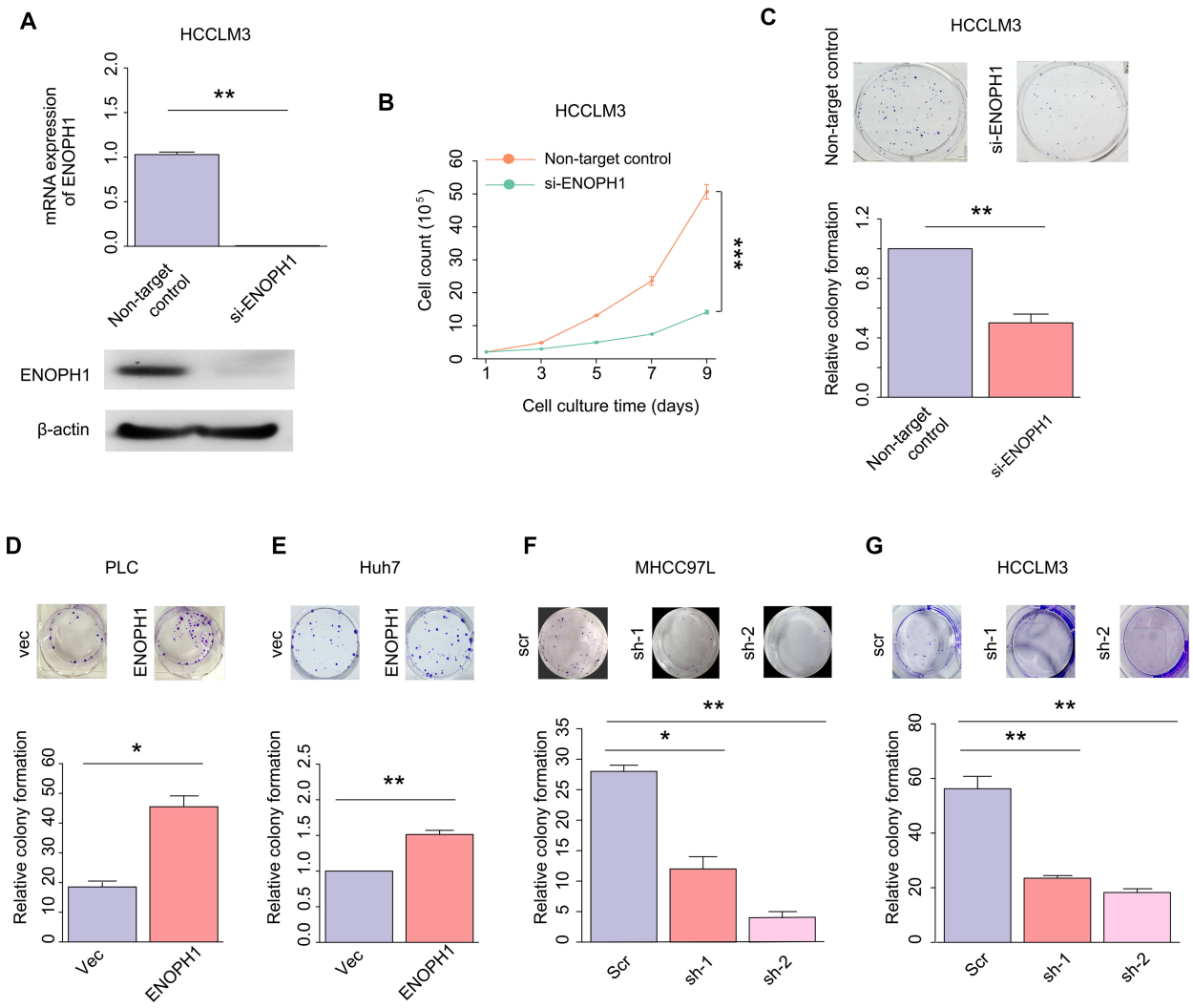


**Fig. S2. ENOPH1 promotes cell proliferation in HCC cells. a** The efficiency of ENOPH1 knockdown with siRNA in HCCLM3 cells by qPCR and Western blot analysis. **b** A growth curve of ENOPH1-knockdown HCCLM3 cells and their corresponding control cells. Representative images show the results of the colony-formation assay in **c** ENOPH1-knockdown HCCLM3 cells, **d** ENOPH1-overexpressing PLC cells, **e** ENOPH1-overexpressing Huh7 cells, **f** ENOPH1-knockdown MHCC97L cells, and **g** ENOPH1-knockdown HCCLM3 cells, and their corresponding control cells (upper panel). Histogram represents colony numbers (below panel). Data represent the means ± SEM (*:$P$ <0.05, **:$P$ <0.01, ***:$P$ <0.001, by Student's $t$-test or two-way ANOVA).

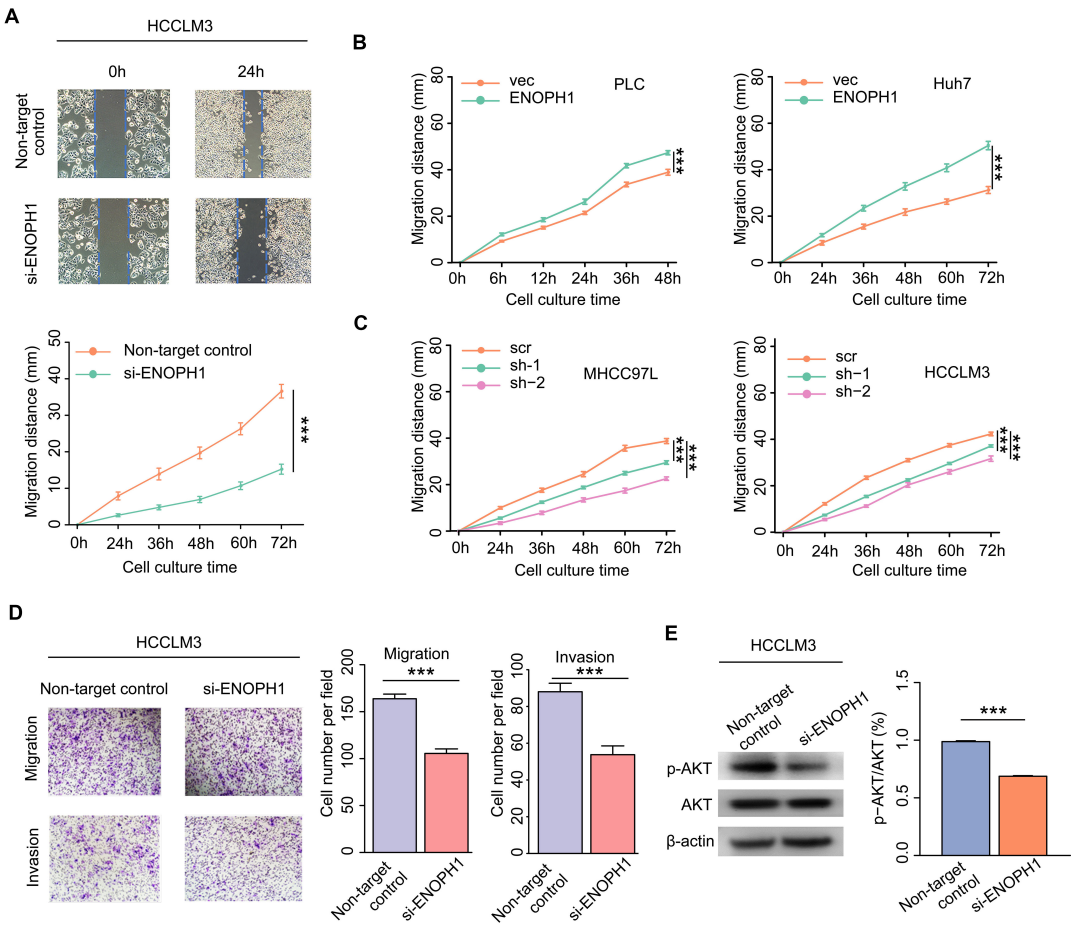


**Fig. S3. ENOPH1 promotes cell migration and invasiveness in HCC cells. a** Wound-healing assays using ENOPH1-knockdown HCCLM3 cells and their corresponding control cells. Representative images were taken at 0 and 48 hours after the scratches were created (upper panel). Line graph is showing migration distance (below panel). **b, c** Line graph showing migration distance in wound-healing assays using ENOPH1-overexpressing HCC cells or ENOPH1-knockdown HCC cells and their corresponding control cells. **d** Transwell-chamber and Matrigel invasion assays using ENOPH1-knockdown HCCLM3 cells and their corresponding control cells. Representative images for the results of the assays. Magnification: 100× (left panel). Histogram shows the the numbers of migrating or invading cells (right panel). **e** Western blots of p-AKT and AKT expression in ENOPH1-knockdown HCCLM3 cells treated and its corresponding control cells (left panel). Histograms shows the relative intensities of p-AKT levels *versus* total AKT expression levels (right panel). Data represent the means ± SEM (*:$P$ <0.05, **:$P$ <0.01, ***:$P$ <0.001, by Student's *t*-test or two-way ANOVA ).

**Supplementary tables**

**Table S1**. Clinicopathological information of the HCC tissue samples for qRT-PCR

| | All | ENOPH1 mRNA relative expression | |
|---|---|---|---|
| | | Low (n=14) | High (n=14) |
| Gender | | | |
| Male | 23 | 11 | 12 |
| Female | 5 | 3 | 2 |
| Age | | | |
| <60 years | 15 | 8 | 7 |
| ≥60 years | 13 | 6 | 7 |
| Serum AFP Level (ng/mL) | | | |
| <400 | 19 | 9 | 10 |
| ≥400 | 9 | 5 | 4 |
| HBsAg | | | |
| Positive | 20 | 10 | 10 |
| Negative | 8 | 4 | 4 |
| Tumor Size | | | |
| <5cm | 10 | 3 | 7 |
| ≥5cm | 18 | 11 | 7 |
| Tumor Number | | | |
| Single | 23 | 11 | 12 |
| Multiple | 5 | 3 | 2 |

Abbreviation: AFP, alpha fetal protein; HBsAg, hepatitis B surface antigen

**Table S2**. Clinicopathological information of the HCC tissue samples for immunohistochemistry

| | All | ENOPH1 tissue expression (%) | | *P* |
|---|---|---|---|---|
| | | Low=90 | High=95 | |
| Age | | | | |
| <60 years | 125 | 58(46.4) | 67(53.6) | 0.377 |
| ≥60 years | 60 | 32(53.3) | 28(46.7) | |
| Gender | | | | |
| Male | 153 | 69(45.1) | 84(54.9) | **0.035*** |
| Female | 32 | 21(65.6) | 11(34.4) | |
| AFP (ng/mL) | | | | |
| <400 | 105 | 61(58.1) | 44(41.9) | **0.003*** |
| ≥400 | 80 | 29(38.9) | 51(63.7) | |
| Child pugh | | | | |
| A | 162 | 83(51.2) | 79(48.8) | 0.062 |
| B | 23 | 7(30.4) | 16(69.6) | |
| Intraoperative hemorrhage(mL) | | | | |
| <300 | 70 | 38(54.3) | 32(45.7) | 0.231 |
| ≥300 | 115 | 52(45.2) | 63(54.8) | |
| Surgery time(min) | | | | |
| <120 | 76 | 36(47.4) | 40(52.6) | 0.771 |
| ≥120 | 109 | 54(49.5) | 55(50.5) | |
| Intraoperative blood transfusion | | | | |
| No | 111 | 63(56.8) | 48 (43.2) | **0.007*** |
| Yes | 74 | 27(36.5) | 47(63.5) | |
| Tumor amount | | | | |
| Single | 146 | 70(47.9) | 76(52.1) | 0.711 |
| Multiple | 39 | 20(51.3) | 19(48.7) | |
| Tumor size(cm) | | | | |
| <5 | 61 | 32(52.5) | 29(47.5) | 0.467 |
| ≥5 | 124 | 58(46.8) | 66(53.2) | |

| | | | | |
|---|---|---|---|---|
| Capsule | | | | |
| Uncomplete | 42 | 18(42.9) | 24(57.1) | 0.393 |
| Complete | 143 | 72(50.3) | 71(49.7) | |
| Microvascular invasion | | | | |
| No | 115 | 62(53.9) | 53(46.1) | 0.066 |
| Yes | 70 | 28(40.0) | 42(60.0) | |
| Macrovascular invasion | | | | |
| No | 145 | 78(53.8) | 67(46.2) | **0.008*** |
| Yes | 40 | 12(30.0) | 28(70.0) | |
| Satellite metastasis | | | | |
| No | 152 | 79(52.0) | 73(48.0) | 0.052 |
| Yes | 33 | 11(33.3) | 22(66.7) | |
| BCLC stage | | | | |
| A | 59 | 31(52.5) | 28(47.5) | 0.235 |
| B | 41 | 15(36.6) | 26(63.4) | |
| C | 28 | 11(39.3) | 17(60.7) | |
| AJCC stage | | | | |
| I+II | 96 | 55(57.3) | 41(42.7) | **0.015*** |
| III+IV | 89 | 35(39.3) | 54(60.7) | |

Abbreviation: AFP, Alpha Fetal Protein; BCLC, Barcelona Clinic Liver Cancer; AJCC, American Joint Committee on Cancer.

* $P < 0.05$

**Table S3**. Sequences of the DNA primers for qRT-PCR.

| Name | Sequence (5'-3') |
|---|---|
| ENOPH1 | Forward: GTAGCTGTGGTGGTGAGACC<br>Reverse: CGGTCTGCCTTAACAACCCT |
| 18s-rRNA (HQ387008.1) | Forward: GCTTAATTTGACTCAACACGGGA<br>Reverse: AGCTATCAATCTGTCAATCCTGTC |

**Table S4**. Sequences of the genes coding siRNA and shRNA for ENOPH1 Knockdown Experiments.

| Name | Sequence (5'-3') |
|---|---|
| ENOPH1-shRNA-1 | GAATGAAGGTGTACATCTATT |
| ENOPH1-shRNA-2 | GACCAGGCAACGCAGGATTAA |
| ENOPH1-siRNA | GGAAGCAGATGTGCACGTA |
| siControl | TCCTAAGGTTAAGTCGCCCTC |

**Table S5**. Details of DEGs in transcriptomic and proteomic data*

| Pathway | DEGs in transcriptomic data | proteomic data |
|---|---|---|
| beta-Alanine metabolism | ALDH1A3 ,AOC3, **CNDP2**, DPYD, GAD1 | **CNDP2** |
| Propanoate metabolism | ACACA, ACACB, BCKDHB, LDHB, PCCB | |
| Purine metabolism | ADCY3, ADCY6, ADCY7, ADPRM, AK2, AK3, AK4, AK7, ENPP4, ENTPD4, ENTPD8, **GART**, GDA, GMPS, HDDC3, HPRT1, NME7, NPR2, NT5C2, NT5E, NT5M, NUDT5, NUDT9, PAPSS1, PDE4C, PDE8A, PDE9A, PFAS, PNP, POLD1, POLE3, POLR2E, POLR3D, PPAT | **GART**, PGM1, POLD1, POLE2, POLR1C, POLR2C, PRPS1 |
| Valine, leucine and isoleucine degradation | AACS, ACADSB, AOX1, BCAT2, BCKDHB, HADH, HADHB, HIBADH, HMGCS1, MCCC2, OXCT2, PCCB | |
| Nicotinate and nicotinamide metabolism | AOX1, BST1, NAMPT, NMNAT1, NNMT, NT5C2, NT5E, NT5M, PNP | |
| Cysteine and methionine metabolism | BCAT2, **ENOPH1**, LDHB, MDH1, MRI1, MTAP, **SDSL**, TST | **ENOPH1**, GCLM, GSS, MDH1, MTAP, **SDSL** |
| Glutathione metabolism | ANPEP, G6PD, GGT5, GGT7, GPX4, GPX8, GSTM1, GSTM2, GSTM4, GSTO2, GSTP1, IDH2, ODC1 | GCLM, GSS, GSTM3, PGD |

*: Bold font indicates that gene expression is up-regulated (or down-regulated) with the metastatic capability at both the mRNA and protein levels.