# Supplementary Materials: Derivate Isocorydine (d-ICD) Suppresses Migration and Invasion of Hepatocellular Carcinoma Cell by Downregulating ITGA1 Expression

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**Table S1.** Antibodies used in this study.

| Antibody | Host | Dilution | Company |
|---|---|---|---|
| ITGA1 | Goat polyclonal | 1:400 for WB | R&D system |
| E2F1 | Mouse mAb IgG2a | 1:100 for WB | Santa Cruz |
| ITGB1 | Mouse mAb IgG2a | 1:200 for WB | Santa Cruz |
| Cleaved-PARP | Rabbit IgG2a | 1:500 for WB | Cell Signalling |
| β -actin | Mouse mAb | 1:10000 for WB | Sigma |

WB: Western blotting.

**Table S2.** The PCR primers for RT-PCR.

| Name | Primer Sequence |
|---|---|
| ITGA1 | Forward: 5′- CAGCAAGAAAGGAGGCATTC -3′ |
| | Reverse: 5′- TTTCCTCGGTTATAGCTGCC -3′ |
| E2F1 | Forward: 5′- CCGTGGACTCTTCGGAGAAC -3′ |
| | Reverse: 5′- ATCCCACCTACGGTCTCCTC -3′ |
| GAPDH | Forward: 5′- AGAAGGCTGGGGCTCATTTG -3′ |
| | Reverse: 5′- AGGGGCCATCCACAGTCTTC -3′ |
| CHIP | Forward: 5′- CGGAGAACGAGATCACCCTCT -3′ |
| | Reverse: 5′- CTCTAAATGCGCTCCACGC -3′ |

**Table S3.** The sequences of ITGA1 ShRNA.

| Name | Primer Sequence |
|---|---|
| ShNC | TTCTCCGAACGTGTCACGT |
| Sh-ITGA1-1 | Forward:5′-CCGGCCCTAGATTCACTAAGACAAA |
| | CTCGAGTTTGTCTTAGTGAATCTAGGGTTTTTG -3′ |
| | Reverse:5′-AATTCAAAAACCCTAGATTCACTAA |
| | GACAAACTCGAGTTTGTCTTAGTGAATCTAGGG -3′ |
| Sh-ITGA1-2 | Forward:5′-CCGGCCCTCGAAACACAACCTTTAACTCGAGTTAAAGGTTG |
| | TGTTTCGAGGGTTTTTG -3′ |
| | Reverse:5′-AATTCAAAAACCCTCGAAACACAACCTTTAACTCGAGTT |
| | AAAGGTTGTGTTTCGAGGG -3′ |
| SiRNA-E2F1-1 | Forward:5′- GGACCUGGAAACUGACCAUTT-3′ |
| | Reverse:5′-AUGGUCAGUUUCCAGGUCCTT-3′ |
| SiRNA-E2F1-2 | Forward:5′- GCAUCUAUGACAUCACCAATT-3′ |
| | Reverse:5′- UUGGUGAUGUCAUAGAUGCTT-3′ |

**Table S4.** Primers for vector constructs.

| Name | Primer Sequence |
|---|---|
| E2F1: | Forward: 5′- GCGGATCCATGGCCTTGGCCGGGG -3′ |
| | Reverse: 5′- CCGGAATTCTCAGAAATCCAGGGGGGTGAGG -3′ |
| ITGA1-promoter | Forward: 5′- GGGGTACCATGGCCTTCAACCTCCTTGGGT -3′ |
| (P1-1822bp) | Reverse: 5′- CCGCTCGAGGTGCTGCCTCACTCCTACT -3′ |
| ITGA1-promoter | Forward: 5′- GGGGTACC CCACCCTTTAACCACGAAT -3′ |
| (P2-830bp) | Reverse: 5′- CCGCTCGAGAGCAGGCGACAGCGACCCCTGG -3′ |
| ITGA1-promoter | Forward: 5′- GGGGTACCTCCTGCCCTGCGAACCAG -3′ |
| (P3-350bp) | Reverse: 5′- CCGCTCGAGGTGCTGCCTCACTCCTACT -3′ |
| ITGA1-promoter | Forward: 5′- GGGGTACC CCACCCTTTAACCACGAAT -3′ |
| (PM-mutant) | Reverse: 5′- CCGCTCGAGCTATAGCCGCAGTTACG |
| | TGTTTAGGCTAAAGTCCACGGGCTGTAGAAGC -3′ |

**Table S5.** ITGA1 promoter sequence.

**>ITGA1 promoter**

-1521 ATGGCCTTCAACCTCCTTGGGTCTGCCCACCCCATTTTTGACGCTCCTTAA
-1470 ACTTCTTCAACTTTTGTCTTCTCCAGCTTTTAGTTGCTGTGATCCTCTATCT
-1418 GGCAATGTATCCAGTGAACCCTGTGGGCTTCTTCCTTCCTGTCCCTCGTG
-1367 TTACCTGTTCTGTGCTACCTGTGTTGCGTTCTGCTCAACGAACCCTCCTTT
-1317 GGGTGGCACTCTTGTCCTGCCTAGCCCTAGTTCTGCTAGAGATGCTCAAT
-1265 TAGTTGTTGAGTTGAAGAATTGAATTGAACTGTTTCCCTCAGATTCCTCTAA
-1213 ACACAAATCCACTTATTCTTTCTGTCTTTGAAATTCTCAAAACCGCTAAGAC
-1162 ACAGCTATTTTGAAAATAGCCAGCATTGGTTGAAGCTAGGGTGAATATGGC
-1112 TTTTGACTCAGAGAGGTTGTTCAACTCACCAGGAAAGGGAAGCATTGCAA
-1060 GTTCCTAAGCACTTCTGGACTCTTAATGCATTTTCACTCGTGAAAGTTTCAA
-1009 GCAAGAAAAAAATAAAGTAGTGCCCCGAGTTCTTTATTAGATTCAATTACC
-958 AGCGTACAAACATCCTAATTCACTTAAACAAAATAGCCCACACACATTCCA
-906 TTTCCAAGGAGGCTGCTTTTCTTCCTCTCTTCCCTTCTCGCTCTCCCTCTTA
-852 CTGTTTTTCCTCCGTCTTGCTTCTCCTTTTCTCCCCTTATCCTAGTTATCTC
-801 AAAGAAAAGCTTATCTGCAACCACCCTTTAACCACGAATTACCATAAACGA
-752 AGCAACCCCCCCACCCCCCCATAAGCGAAAGCAAAGCCCTCAGTGGAGC
-703 CTGTCGGGGTGGTGGGGACGCCCCCTTTCGTGCAAAACGACTTCACGGT
-654 GAATTTCAAGTGTCCGCAGGGGATGGAAGGGGGGATTCCAGATCCTCGC
-604 ATTTTCACAGACGGCCTAATCCCTCTGCGCAATGGACCCGGGAAAGCTGC
-555 CTCCCGTTAAGGGCGCCCAGGCACTGTCAAAGCGCCCTCCCTGCCCGGG
-507 CCGGGAGAAGTAAGGGGTGTGAAGGCGGAGCCACTGGGCTGGCAACAC
-457 CCTCCCGAAGGTCCAGCCCTTACCCGCCTTCCTACCACCTTAGGGGATTT
-407 GGCCCGGAGAACGAGATCACCCTCTCAATGAAAGGCAGATGTCCCTTTAA

E2F1 binding site

-357 GGTTTGCTTCTACAGCCCGTGGACTTTAGCCTAAACACGGACCCGCGAAG
-307 CTGGCTTTATTTGTCCATGTCTCGGACAGAGCCTGGGAAGCTGCCAGTGA
-258 GATTTCAGAGACCAAGAGCGCGAAGGGGCGGGCGATGTGGCAATCCGTC
-208 TGGGATGTGAAAAGCGTGGAGCGCATTTAGAGGAATTCGACGAAAACACA
-159 GGAAATCACTCCTCTCCCGCTCCTGGGCGCCGCTGCCACTGGGGCAGAG
-111 GACTGGGAACCGCGGCAGCGGGATAAGTGGCCCAGCCAGAGAGCGCAG
-63 CTCCCGCGCCCGGTCCTGCCCTGCGAACCAGCGCGGCCCCCTGGCGCT

Transcriptional initiation site

-15 GAGGCTGCTCCGGCCATGGCCCCTCGGCCCCGCGCCCGCCCAGGGGTC
+34 GCTGTCGCCTGCTGCTGGCTCCTCACTGGTGAGCGACTCGCTTTTCTCTG
+84 AGCATCTCCTGCTCGCGGGCTTGGGGCTTGGAGCGGGGAGGGAGGTCCT

| |
|---|
| +134 CAGAGCCATGGGCCAGATAGGAAGAGAGAGCGCCCATCCACTTTCGGGC |
| +182 ATTCCAGGTACTCAGGCCAGGCTACGGGGCAGGCAGGAGTTTCGCGTTC |
| + +232 TGGTTTTGATATGAATTAGTTTTCGTGTCTCTCCAAAGTCCTTCACCCTAGG |
| +283 AGTAGGAGTGAGGCAGCAC |

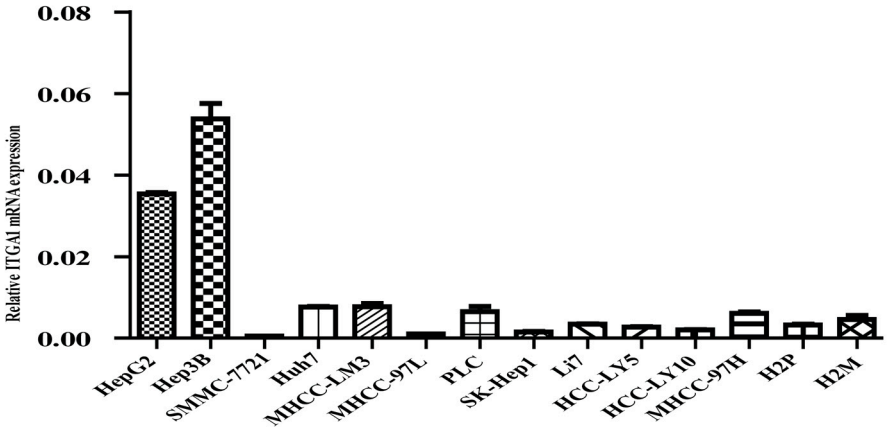


**Figure S1.** RT-PCR analyzed endogenous ITGA1 mRNA expression in various hepatocellular carcinoma (HCC) cell lines.

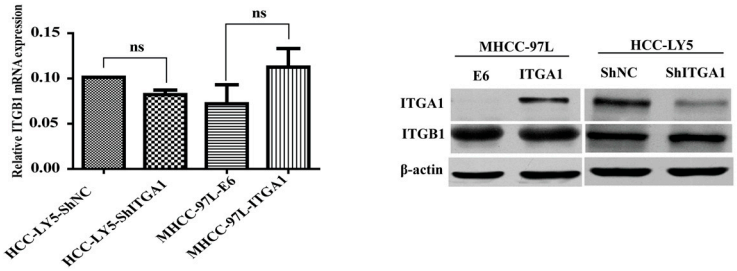


**Figure S2.** The mRNA and protein level of ITGB1 in ITGA1 overexpression HCC cells and ITGA1 silencing HCC cells were detected by RT-PCR and western blot. Results showed that the expression change of ITGA1 have no impact on ITGB1 in HCC cells. ("ns" indicates no statistical significance).

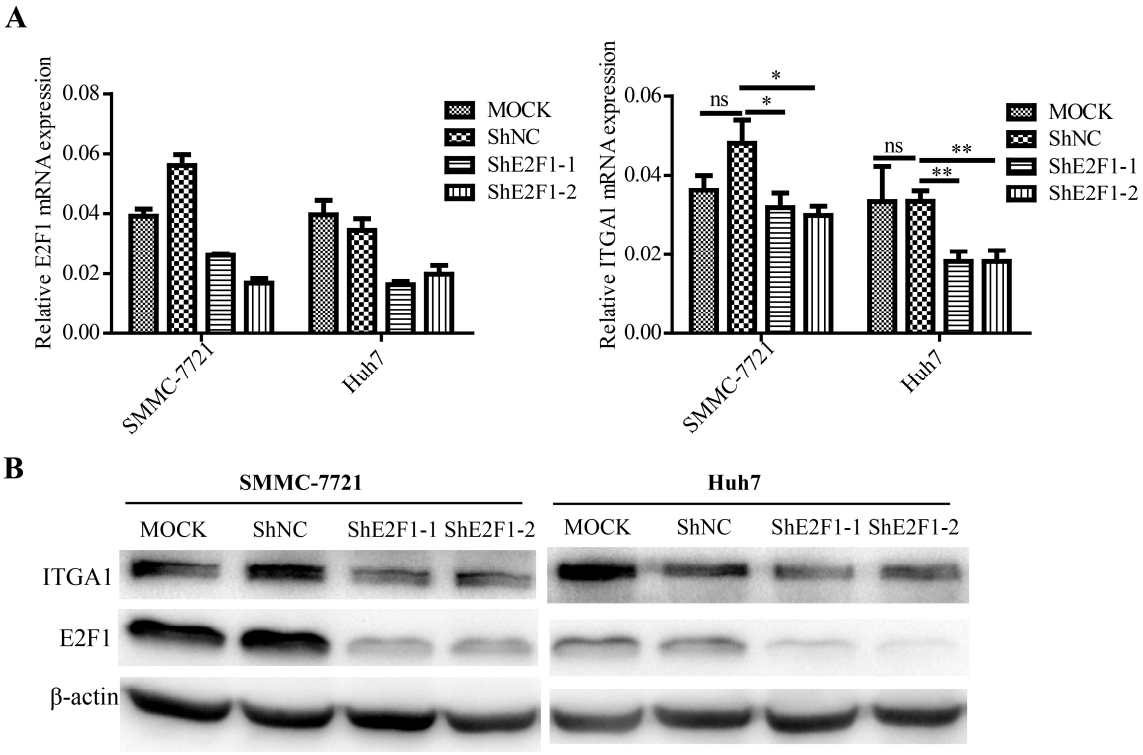


**Figure S3.** (**A**,**B**) RT-PCR and western blotting analyzed E2F1 and ITGA1 expression in HCC cells with transiently transfected E2F1 SiRNA. ("ns" means no significance, * $p < 0.05$, ** $p < 0.01$).

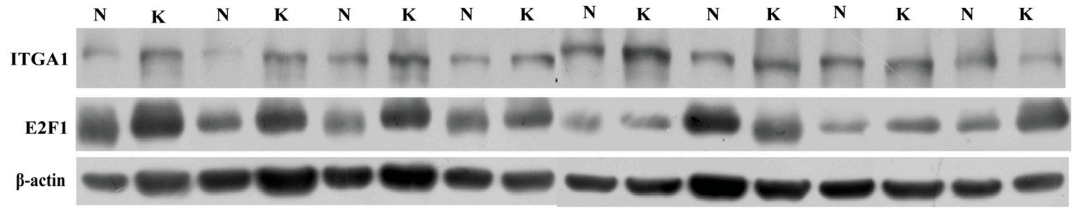


**Figure S4.** Western blot analyzed the expression of ITGA1 and E2F1 in HCC tissue. The result showed that the expression of ITGA1 positive related to the expression of E2F1 in HCC.

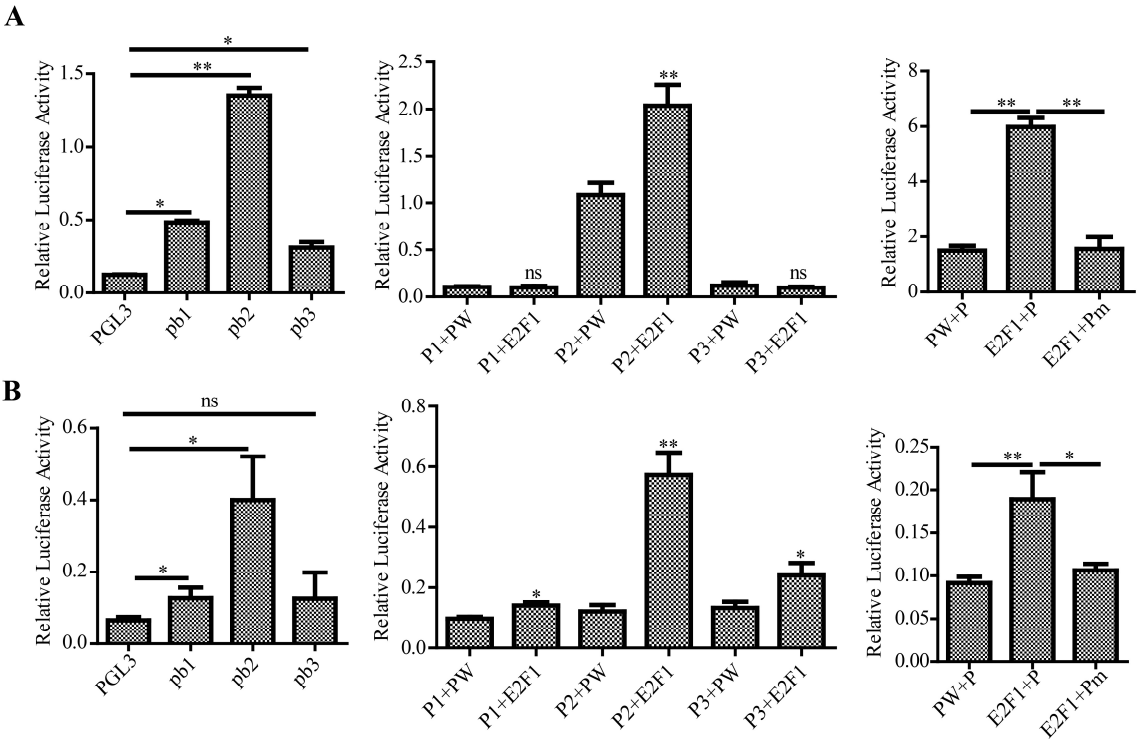


**Figure S5.** E2F1 binds to ITGA1 promoter in HCC cells. (**A**,**B**) Dual luciferase reporter gene studies were performed to detect the ITGA1 promoter and its truncated and mutant construct activity in SMMC-7721 and Huh7 cells. ("ns" means no significance , * $p < 0.05$, ** $p < 0.01$).

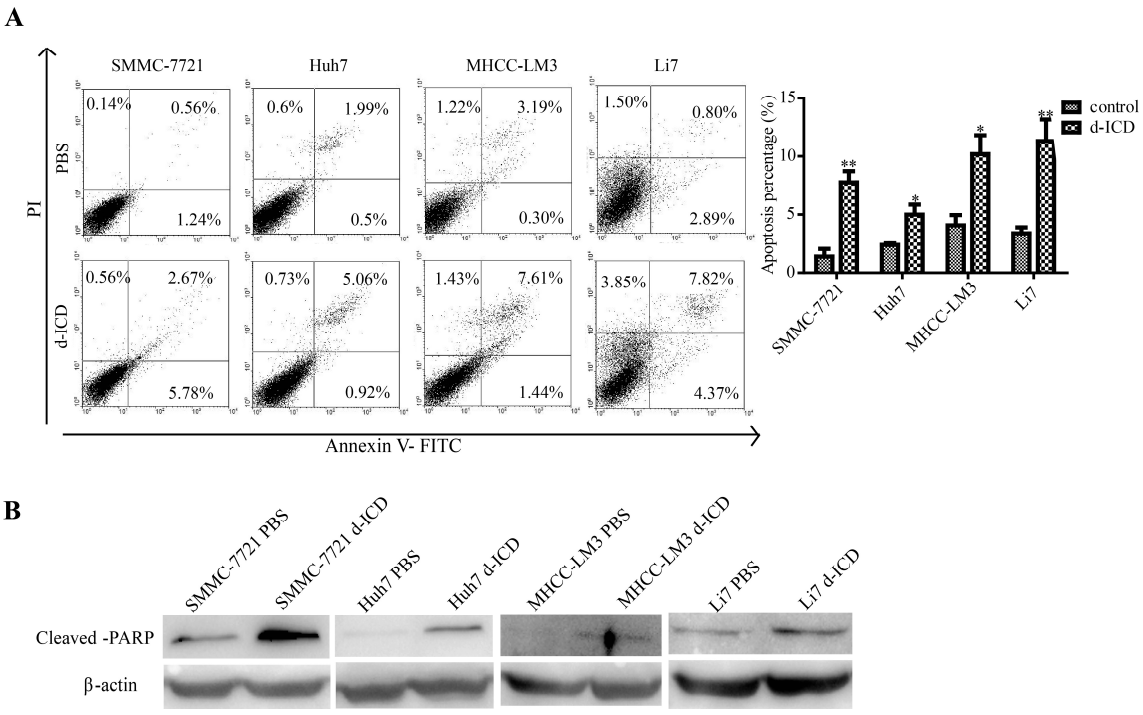


**Figure S6.** Derivate isocorydine (d-ICD) mildly induce HCC cell apoptosis during migration assay. (**A**) Following the treatment of SMMC-7721, Huh7, MHCC-LM3, Li7 cells with d-ICD for 48h, cells were stained by Annexin V-FITC and PI, and apoptosis cells were detected by FACS analysis. (**B**) Western blot analysis of cleaved PARP in SMMC-7721, Huh7, MHCC-LM3, and Li7 cells following d-ICD treatment 48h (* $p < 0.05$, ** $p < 0.01$).

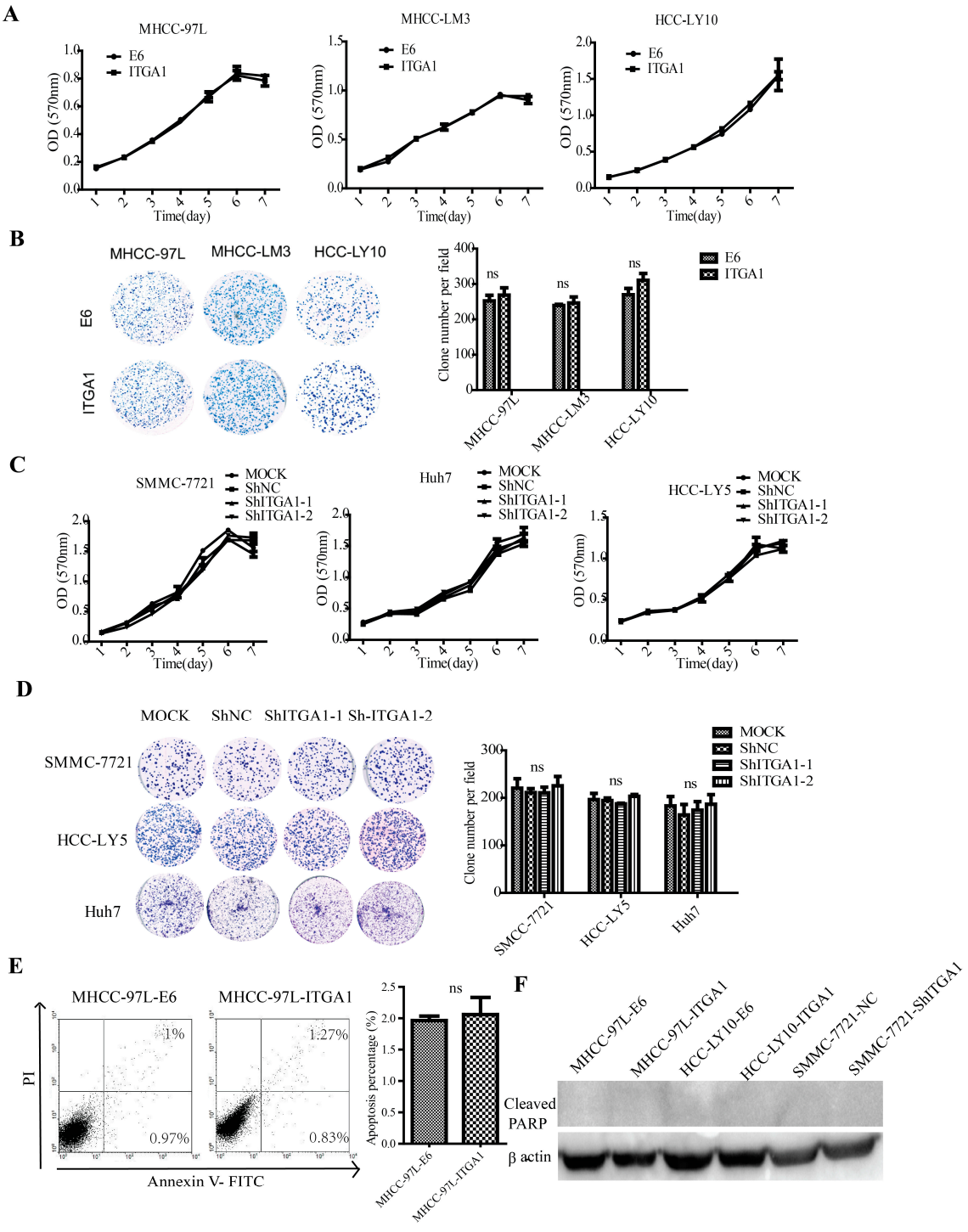


**Figure S7.** ITGA1 has no obvious effect on HCC cells proliferation or apoptosis in vitro. (**A**,**B**) MTT assay and clone formation assay were performed to analyze ITGA1 over-expression HCC cell proliferation ability, and the statistical analysis showed no significance between the two group. (**C**,**D**) MTT assay and clone formation assay were performed to analyze ITGA1 silencing-expression HCC cell proliferation ability, and the statistical analysis showed no significance between MOCK, ShNC, and ShITGA1 group. (**E**) Annexin v-FITC and PI double staining and FACS analyzed apoptosis cells in ITGA1 overexpression MHCC-97L cell and the control group cell, no difference was observed. (**F**) Western blot analysis of cleaved PARP in MHCC-97L and HCC-LY10 with ITGA1 overexpression and SMMC-7721 with ITGA1 silencing ("ns" means no significance).