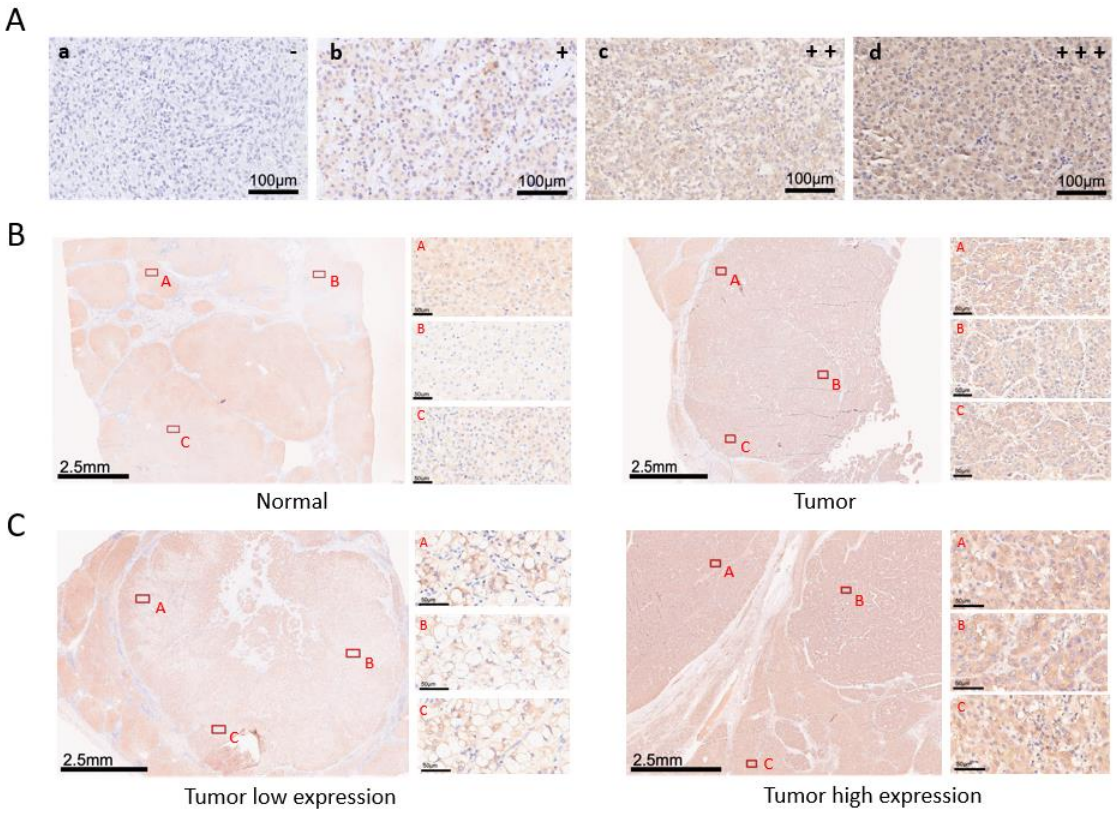
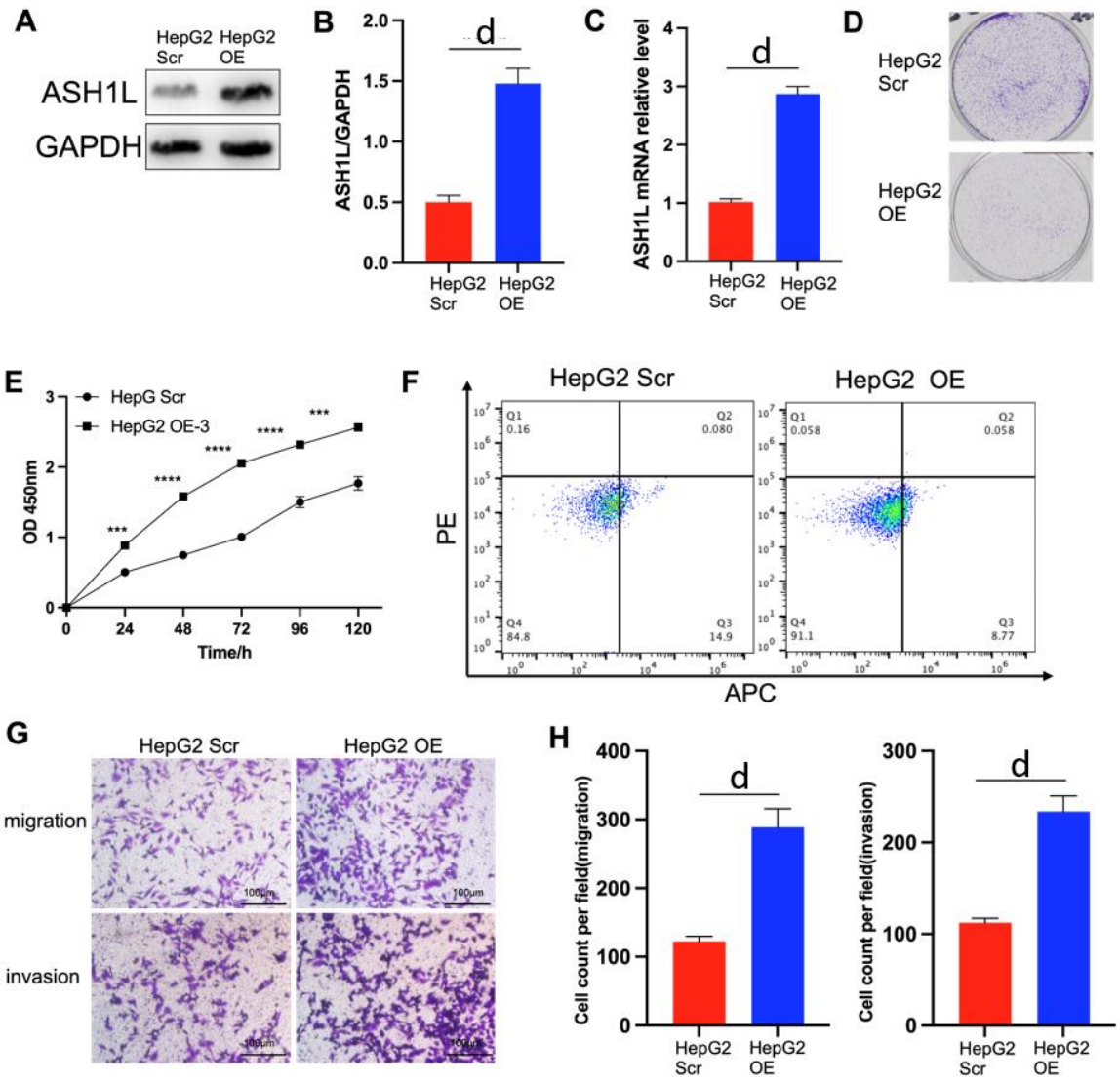
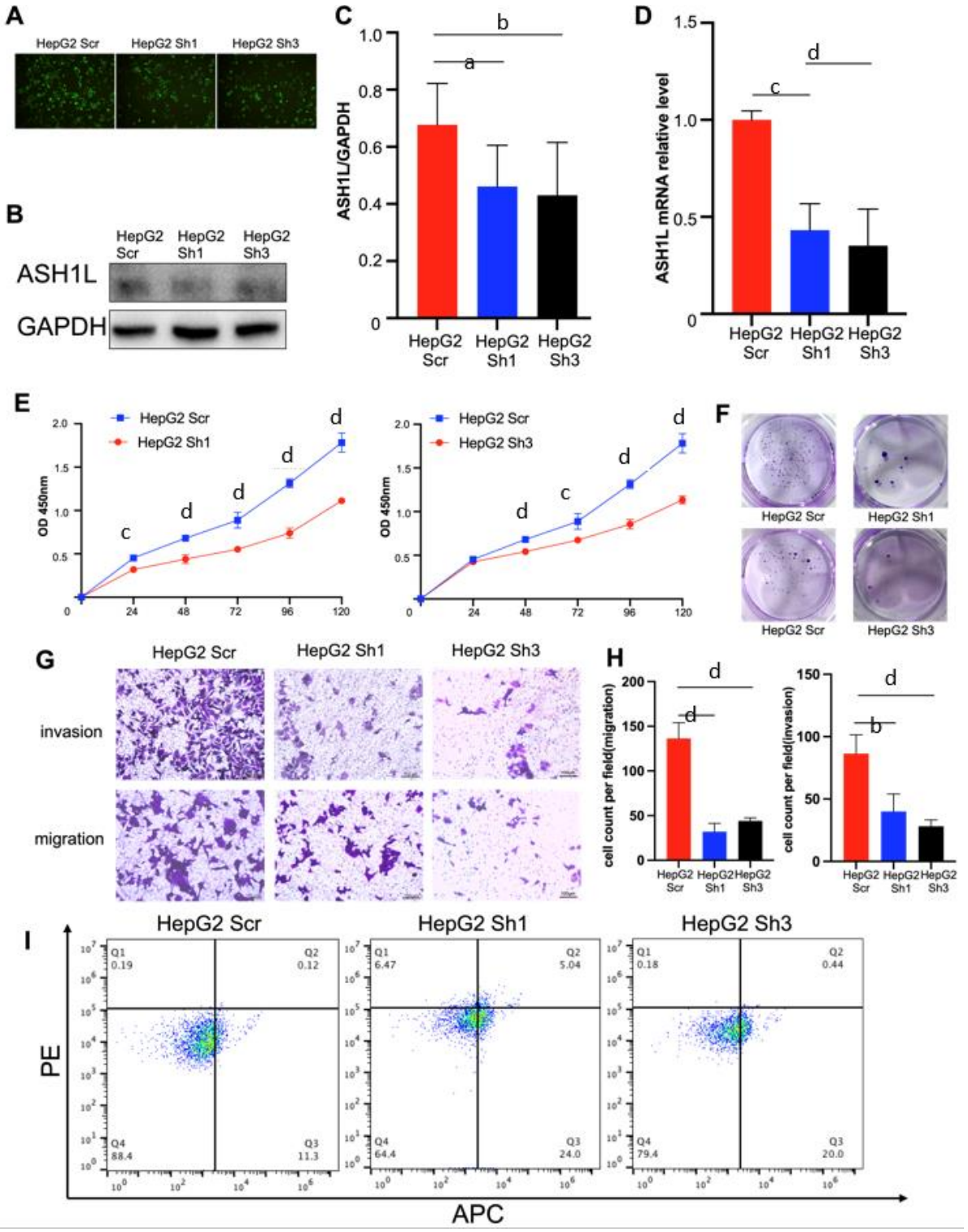


Supplementary Figure 1 ***ASH1L*** **protein with various expression levels in patient tissues. A:** Immunohistochemical Staining of *ASH1L* in HCC (Immunohistochemical staining was performed using a semi-quantitative integral method based on overall staining intensity and percentage of positive cells. The results were classified as follows: A. Negative (-); b. Weak positive (+); c. Moderately positive (++); d. Strong positive (+++). Magnification: 400 ×); **B:** Representative Images of *ASH1L* Expression in Normal Liver and HCC Tissues from the Same Patient; **C:** Representative Images Showing Weak *ASH1L* Expression in the Tumor Low Expression Group and Strong *ASH1L* Expression in the Tumor High Expression Group.

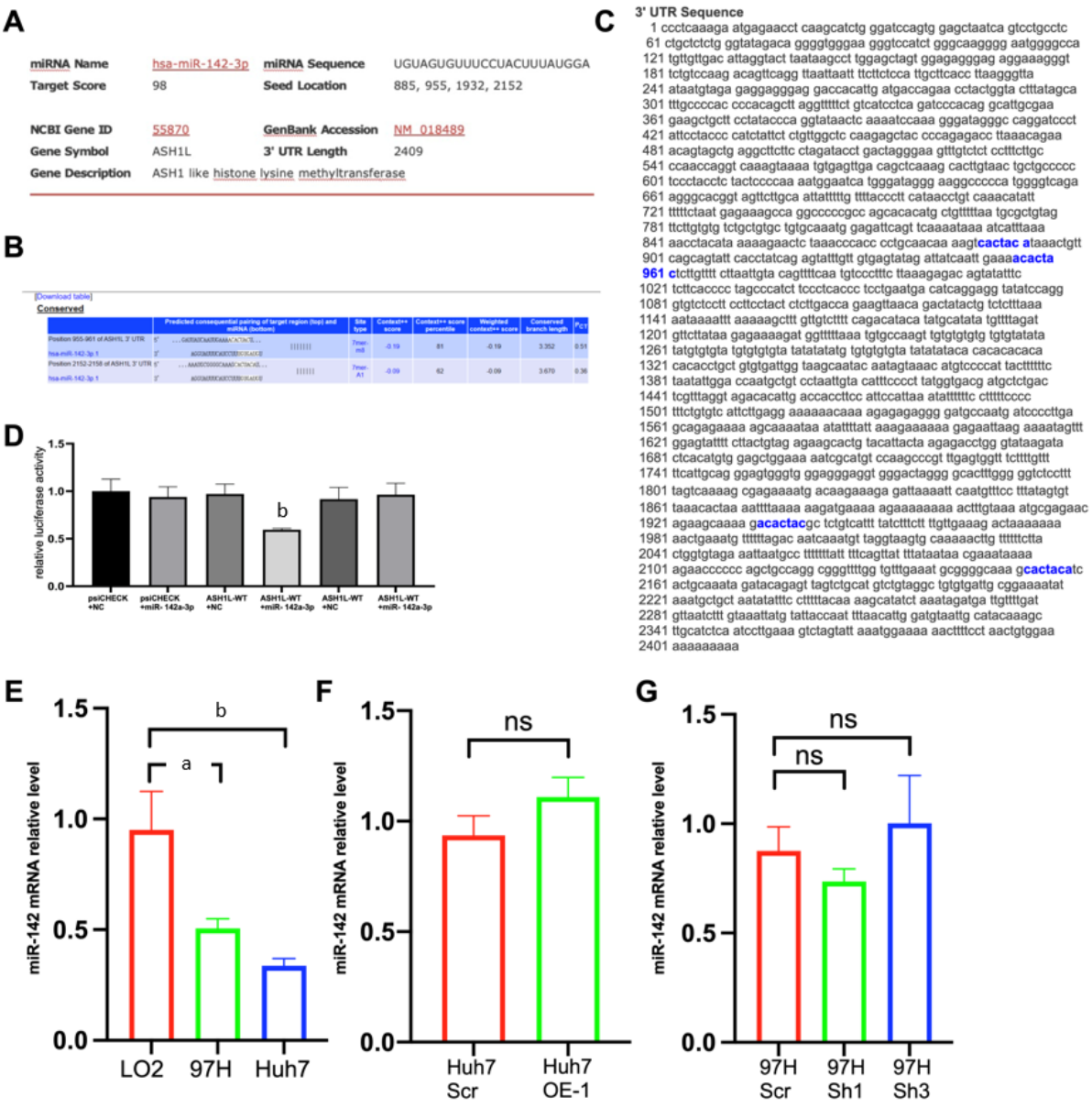


Supplementary Figure 2 *ASH1L* expression in HepG2 and OE cells, and proliferation, migration, invasion and apoptosis assay of HepG2-ASH1L-OE. A: Western blot results of HepG2 Scr and OE cells; B: Quantitative results of A; C: RT-qPCR analysis comparing ASH1L expression between HepG2 Scr and OE cells; D: The colony formation assay between HepG2 Scr and OE cells; E: CCK-8 assays results between HepG2 Scr and OE cells; F: Cell apoptosis was detected by flow cytometry, and the results showed the influence of ASH1L over expression on apoptosis; G: Transwell chamber cell migration and invasion results of HepG2 Scr and OE cells; H: Quantitative results of E after independently repeated 3 times. $^{d}P < 0.0001$.



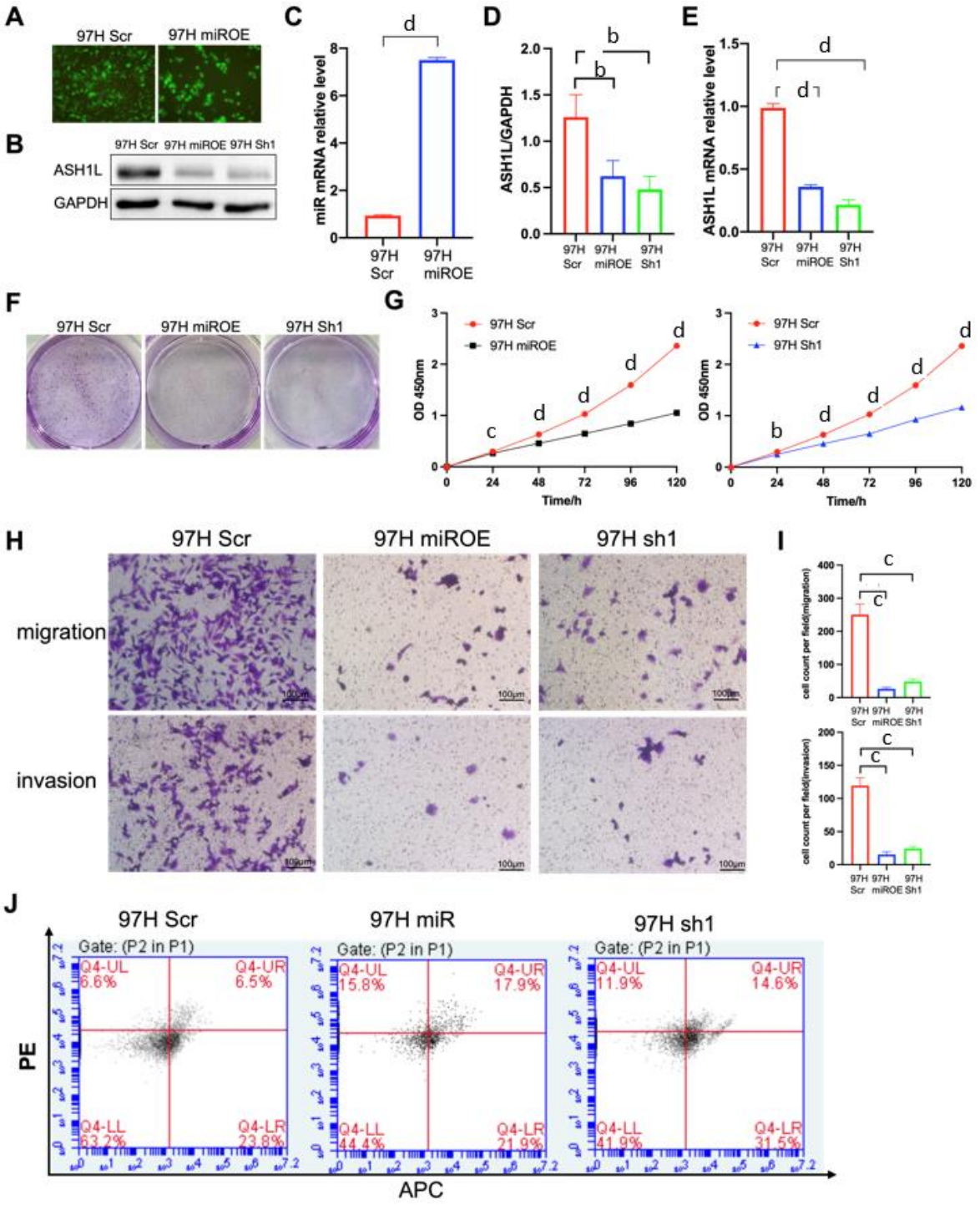
Supplementary Figure 3 *ASH1L* expression in HepG2 Scr and KD cell lines and their proliferation, invasion, migration, and apoptosis assays. A: Fluorescence imaging was conducted post-lentiviral transfection to visualize the *ASH1L* protein levels; B: Western blot analysis was performed to compare the protein expression between HepG2 Scrambled (Scr) and Knockdown (KD) groups. Western blot of *ASH1L* expression in HepG2 Scr and KD cells; C: Quantitative results of B; D: RT-

qPCR analysis of *ASH1L* expression in HepG2 Scr and KD cells; E: CCK-8 assay results; F: Colony formation assay results; G: Transwell chamber test cell migration and invasion results of HepG2 Scr and KD cells; H: Quantitative results of G; I: Cell apoptosis was detected by flow cytometry, and the results showed the influence of *ASH1L* knock down on apoptosis. [a]$P < 0.05$. [b]$P < 0.01$. [c]$P < 0.001$. [d]$P < 0.0001$.



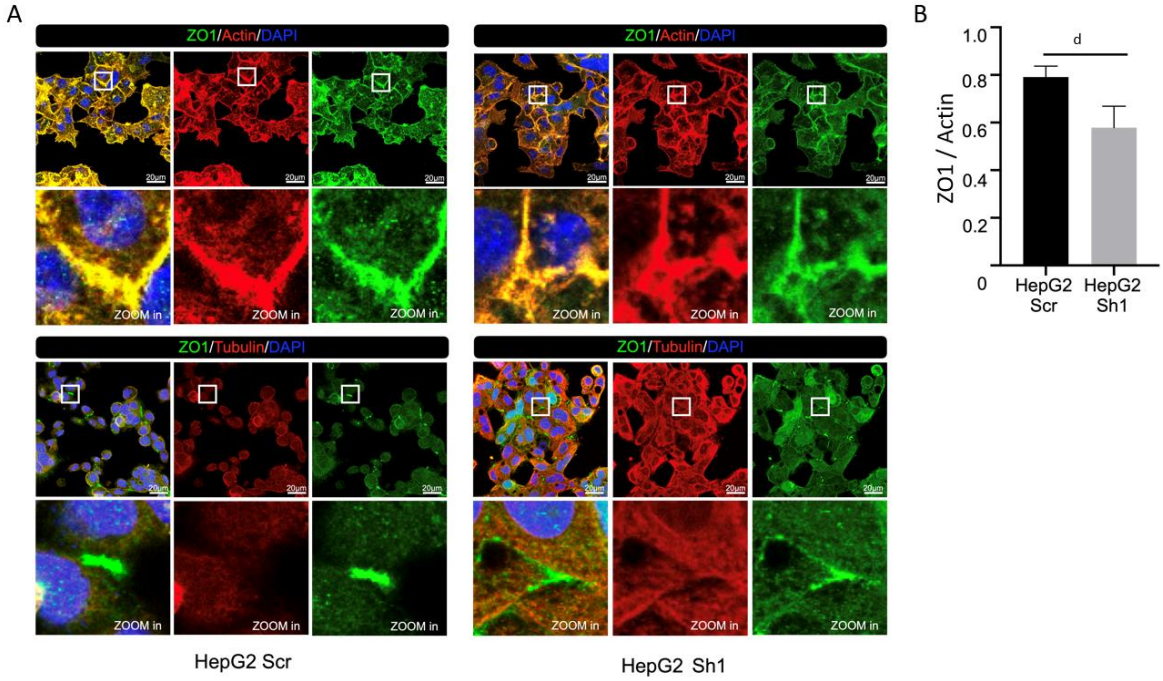
Supplementary Figure 4 Contents of miR-142-3p among cell lines. A: MicroRNA and Target Gene Description; B: Results predicted by Targetscan; C: Dual luciferase locus: 955-961 ASH1L- 3' UTR; D: The ratio of miR- 142-3p to ASH1L-WT cotranscriptional dual luciferase activity was significantly down-regulated ($P < 0.05$)

compared with the control group, and miR- 142-3p could target the ASH1L 3'UTR; E: Contents of miR-142-3p among liver cell and HCC cell lines by RT-qPCR; F: RT-qPCR analysis between Huh7-ASH1L-OE cell lines; G: RT-qPCR analysis among MHCC-97H Scr and KD groups. [a]$P < 0.05$. [b]$P < 0.01$.



Supplementary Figure 5 The expression of *ASH1L* and proliferation assay, migration and invasion assay in MHCC-97H, miR and KD cell lines. A: Fluorescence

imaging after transfection of lentivirus; B: Western blot analysis among MHCC-97H Scr, miR and KD groups; C: RT-qPCR analysis among MHCC-97H Scr, miR and KD groups; D: Quantitative results of B; E: RT-qPCR analysis among MHCC-97H Scr, miR and KD groups; F: Colony formation assay among MHCC-97H Scr, miR and KD groups; G: CCK-8 assays among MHCC-97H Scr, miR and KD groups; H: Transwell chamber tests were used to show the different abilities of cell migration and invasion among MHCC-97H Scr, miR and KD groups; I: Quantitative analyses of H; J: Cell apoptosis was detected by flow cytometry, and the results showed the influence of miR-142-3p over expression on apoptosis. $^{b}P < 0.01$. $^{c}P < 0.001$. $^{d}P < 0.0001$.



Supplementary Figure 6 ZO1 expression at cellular junctions in HepG2 Scr and KD cells. A: IF staining for ZO1 content in HepG2 Scr and KD cells; B: Quantitative results of A. (ZO1 and Actin are expressed inside cells and at cell junctions, while Tubulin is expressed exclusively inside cells. $^{d}P < 0.0001$.

Supplementary Table 1 Lentivirus sequences

Serial Number	Sequences
CMV-F	CGCAAATGGGCGGTAGGCGTG
WPRE-R	CATAGCGTAAAAGGAGCAACA
Sg10724	AGGAATGGAACAGTCCCGCG
Sg10725	GCCTCCCACAATCCCCCCCG
Sg10726	CAGTCCCGCGGGGGGGATTG
Sh1	TGCTGTTGGAGAGCGATATAACTCGAGTTATATCGCTCTCCAACAGCATTTTT
Sh2	CCTGCCAAATACCATAAGAAACTCGAGTTTCTTATGGTATTTGGCAGGTTTTT
Sh3	CCCTGAAACTTCTTCACAGTTCTCGAGAACTGTGAAGAAGTTTCAGGGTTTTT
miR	CGCAAATGGGCGGTAGGCGTG