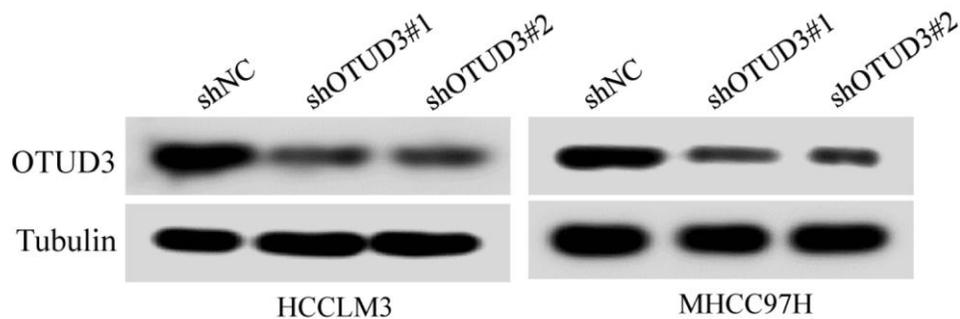
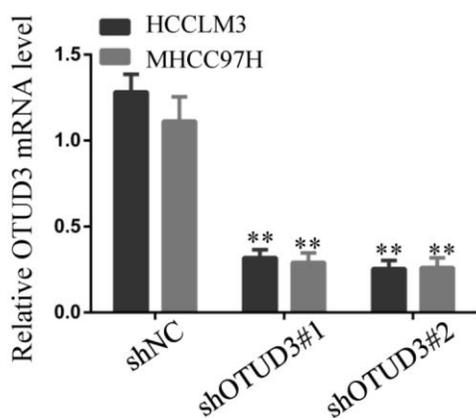


SUPPLEMENTARY FIGURES

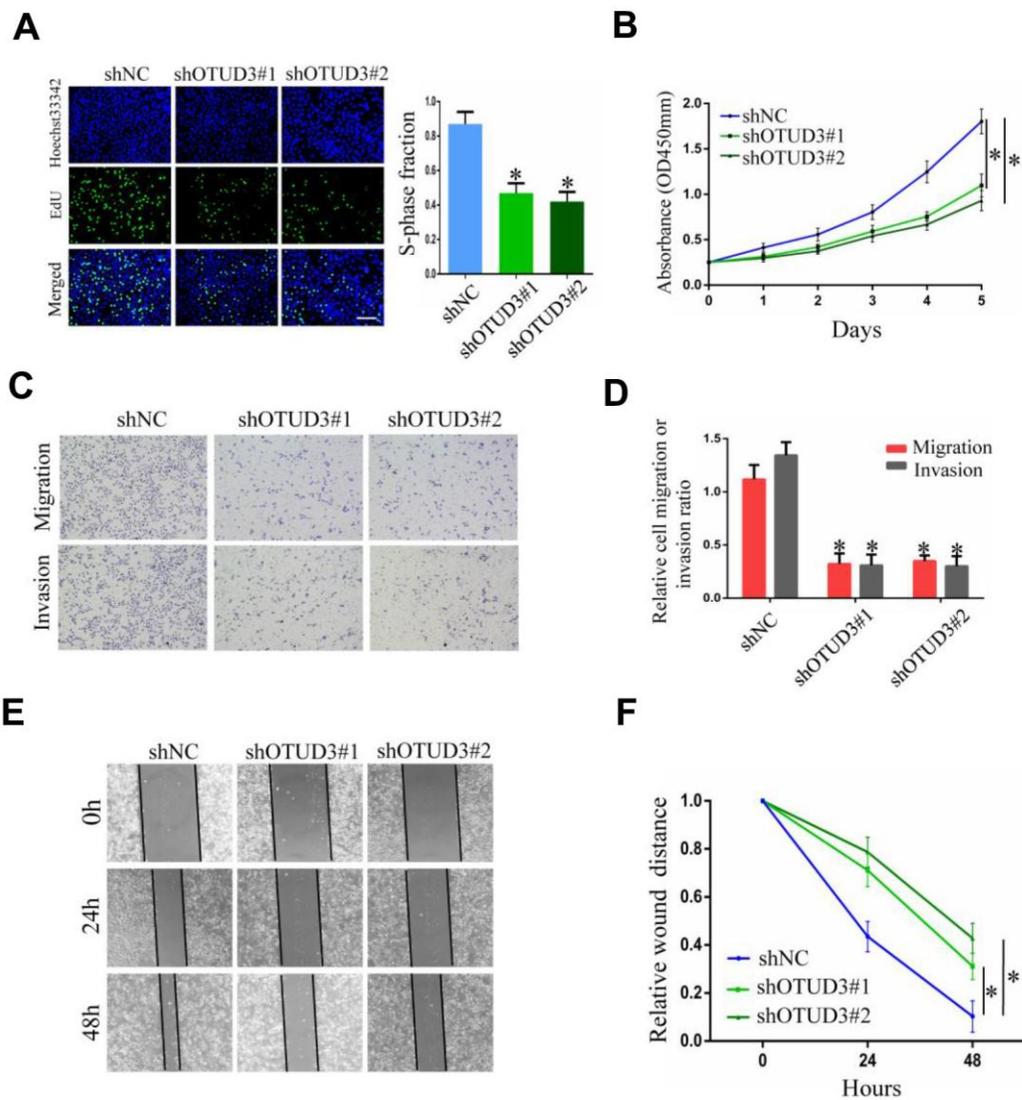
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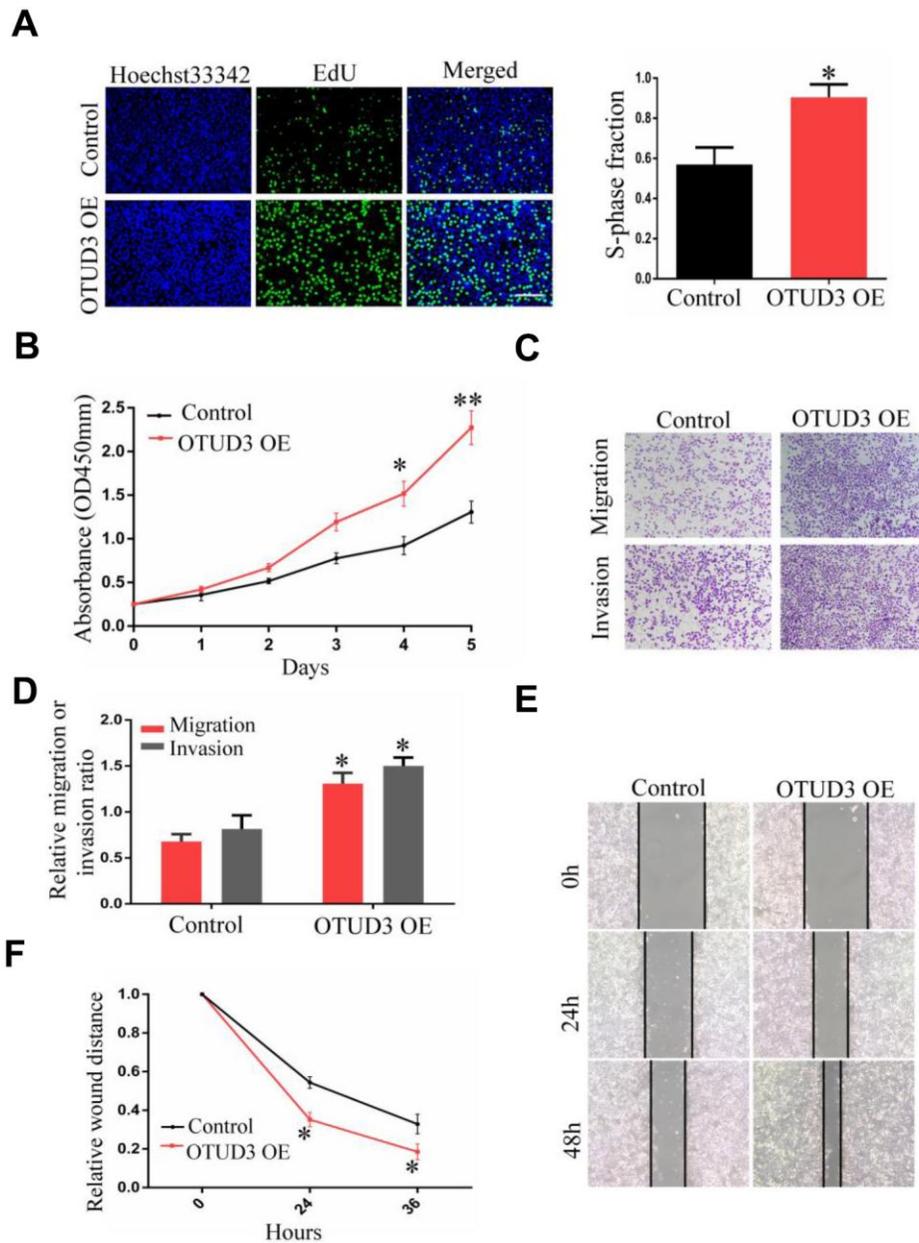
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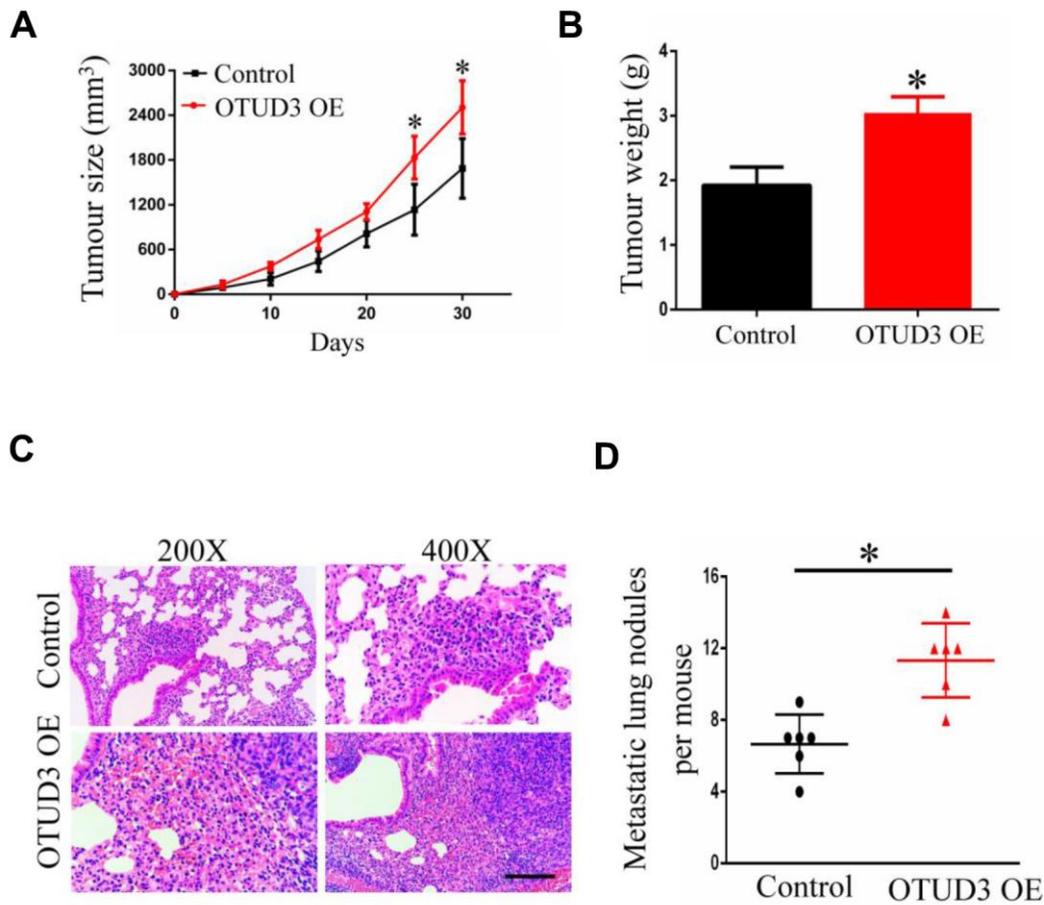
Supplementary Figure 1. OTUD3 shRNA transfection efficiency. (A, B) The OTUD3 knockdown efficiency was confirmed by western blot and qRT-PCR analysis. Data were mean \pm S.D. of three independent determinations. ** $P < 0.01$ of t-tests.



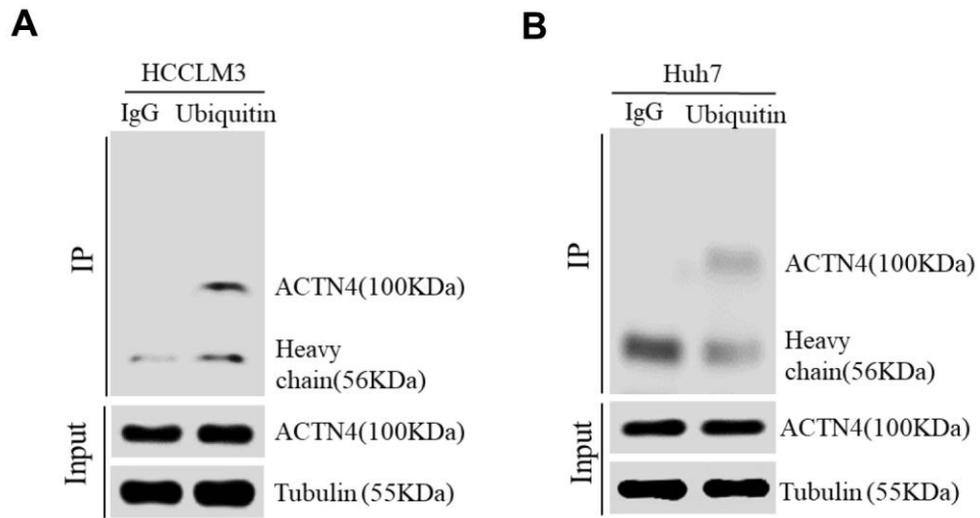
Supplementary Figure 2. OTUD3 suppression inhibits HCC cells proliferation, migration and invasion. (A) EdU assay evaluating the proliferation ability for HepG2 cells transfected with control shRNA or shRNA targeting OTUD3. Right panel is quantification of the results of the EdU assay. (B) CCK8 examining the effect of OTUD3 knockdown on the proliferation of HepG2 cell. (C, D) Invasion and migration assays were employed to evaluate the effect of OTUD3 knockdown on HepG2 cells metastatic ability (Magnification 200X). (E, F) Scratching assay was performed to detect migration ability of OTUD3 knockdown HepG2 cells compared with the control group.



Supplementary Figure 3. OTUD3 overexpression facilitates HCC cells proliferation, migration and invasion *in vitro*. (A–C), EdU assay and CCK8 experiments showing cell proliferation capacity of OTUD3 overexpressing Huh7 cell compared with the control group. (D–F) Transwell migration and invasion assay and wound healing assay evaluating the metastatic ability of OTUD3 overexpressing Huh7 cell compared with the control group. Data were mean \pm S.D. of three independent determinations. * $P < 0.05$, ** $P < 0.01$ of t-tests.



Supplementary Figure 4. OTUD3 overexpression accelerates HCC cells proliferation, migration and invasion *in vivo*. (A, B) Tumour sizes and tumour weights of the control or Huh7-OTUD3 OE groups (n=6 per group) of nude mice were measured and corresponding tumour growth curves were obtained. (C) Images of H&E staining of paraffin-embedded lung tissues from the control or Huh7-OTUD3 OE nude mice group (n=6 per group) (Magnification: 200X, 400X; scale bar: 50 μ m). (D) Quantification of metastatic lung nodules with the control or Huh7-OTUD3 OE cells by tail-vein injection. Data were mean \pm S.D. of three independent determinations. * $P < 0.05$ of t-tests.



Supplementary Figure 5. ACTN4 bind ubiquitin directly in HCC cells. (A, B) co-IP experiments between endogenous ubiquitin and ACTN4 in HCCLM3 and Huh7 cells. ACTN4 was detected in the immunoprecipitation when the anti-ubiquitin antibody was used as bait.