

Supplementary Figure Legend

Figure S1 Hepatoma cells stably expressing USP27 knockdown or overexpression constructs were generated and the interactions between Cyclin E and DUBs were determined. (A-C) Western blot analysis of USP27 expression in Hep3B (S1A, S1B) or MHCC97H (S1C) cells was performed. GAPDH was used as loading control. (D) USP27 or USP28 expression plasmids were transfected alone or cotransfected with Cyclin E into HEK293T cells and their interaction was determined by immunoprecipitation and immunoblot. (E) The interaction between Cyclin E and USP15 or USP27 was determined as in (D).

Figure S2 Flow cytometry analysis of Hep3B cells with USP27 knockdown or overexpression. (A-D) Hep3B cells were synchronized at G2/M by treating with 50ng/ml nocodazole for 16h. The cells were collected at the indicated time points following the removal of nocodazole. The cells were analyzed by FACS (A-C) and the relevant mRNA level were determined (D). (E-G) Flow cytometry analysis was performed to determine the cell cycle progression when USP27 was depleted (E, F) or overexpressed (G). (H-I) MTS assay was performed to determine the cell proliferation when USP27 was knocked down in Hep3B (H) or MHCC97H cells (I).

Figure S3 The E3 ubiquitin ligase Fbxw7 downregulates USP27 expression. (A) Fbxw7 expression plasmid was transfected alone or cotransfected with USP27 or Cyclin E into HEK293T cells and their interaction was determined by immunoprecipitation and immunoblot. (B) Fbxw7 expression plasmid was transfected into HEK293T cells. Cells were treated with CHX as indicated. USP27 protein stability in the transiently transfected HEK293T cells was examined as described in Figure 3A. (C) The working model among Cyclin E, USP27 and Fbxw7 was shown.

Figure S4 USP27 knockdown inhibits colony formation. (A&B) Clonogenic assay

was performed to measure the capacity of colony formation in USP27-depleted hepatoma cells. 1×10^3 Hep3B (A) or MHCC97H (B) cells were seeded in 6-well-plate to form colonies in 3 weeks and colonies with no less than 50 cells per well were counted. Quantitation of colony number was shown in the right panel.

Figure S5 Downregulation of USP27 suppresses hepatocellular migration and metastasis. (A&B) Knockdown of USP27 decreased Hep3B cell migration as measured by wound-healing assays. Scale bar: 100 μ m. Results presented represent the mean of triplicate experiments $\pm$ SEM. * $P < 0.05$. **(C&D)** The effect of USP27 knockdown on MHCC97H cells migration was measured as in (A&B). **(E-G)** The effects of USP27 knockdown (E&F) or over expression (G) on cell invasion in Hep3B cells or MHCC97H cells were measured by Matri-gel transwell assays.