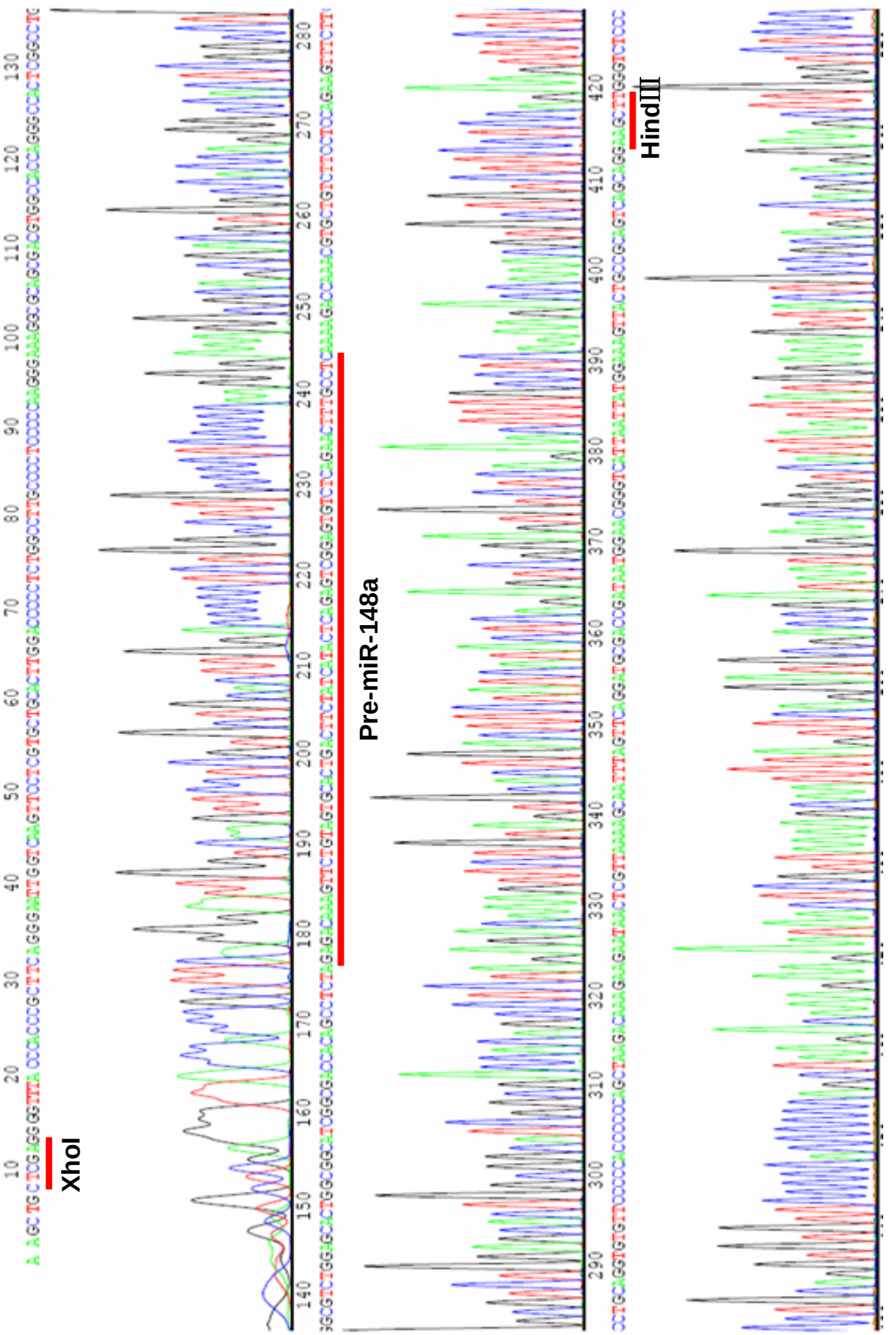
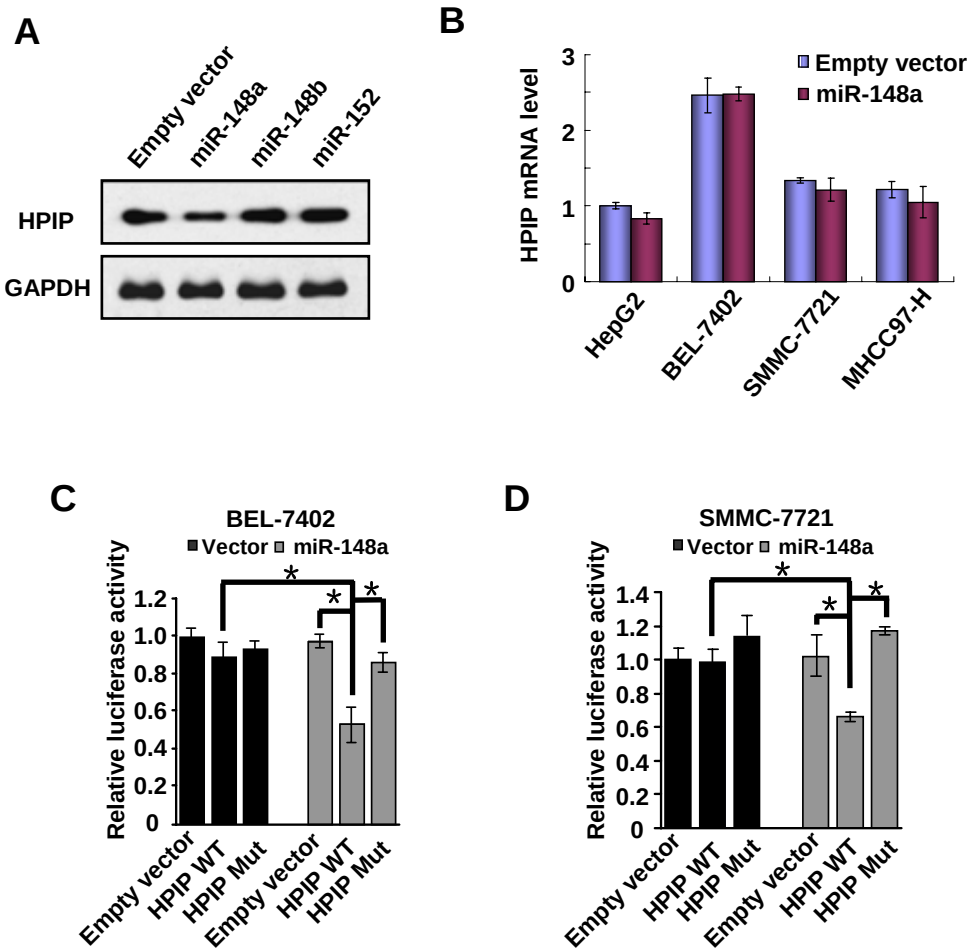
Supplemental Figure S1



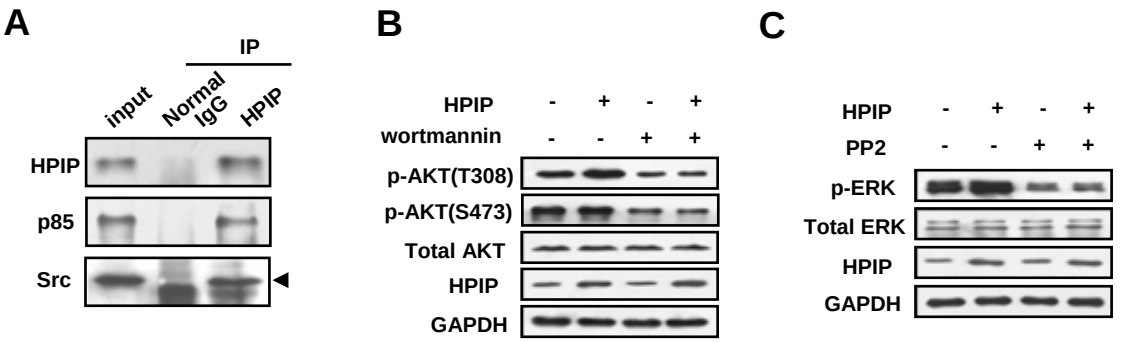
Supplemental Figure 1. DNA sequencing of miR-148a cloned into pcDNA3.0 vector. The pre-miR-148a sequence is underlined. The cloning sites are XhoI and HindIII as indicated.

Supplemental Figure S2



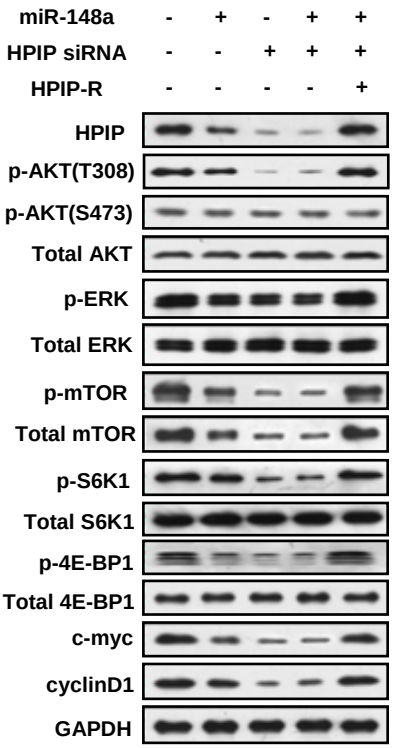
Supplemental Figure 2. MiR-148a inhibits HPIP expression at the protein level. (**A**) Immunoblot analysis of HepG2 cells transfected with miR-148a, miR-148b or miR-152. (**B**) HepG2, BEL-7402, SMMC-7721 and MHCC97-H cells transfected with miR-148a or empty vector were used for real-time RT-PCR with HPIP primers. (**C** and **D**) MiRNA luciferase reporter assays in BEL-7402 (C) and SMMC-7721 (D) cells transfected with miR-148a and wild-type or mutated HPIP reporter. All values shown are mean ± SD of triplicate measurements and have been repeated 3 times with similar results (*p < 0.01).

Supplemental Figure S3



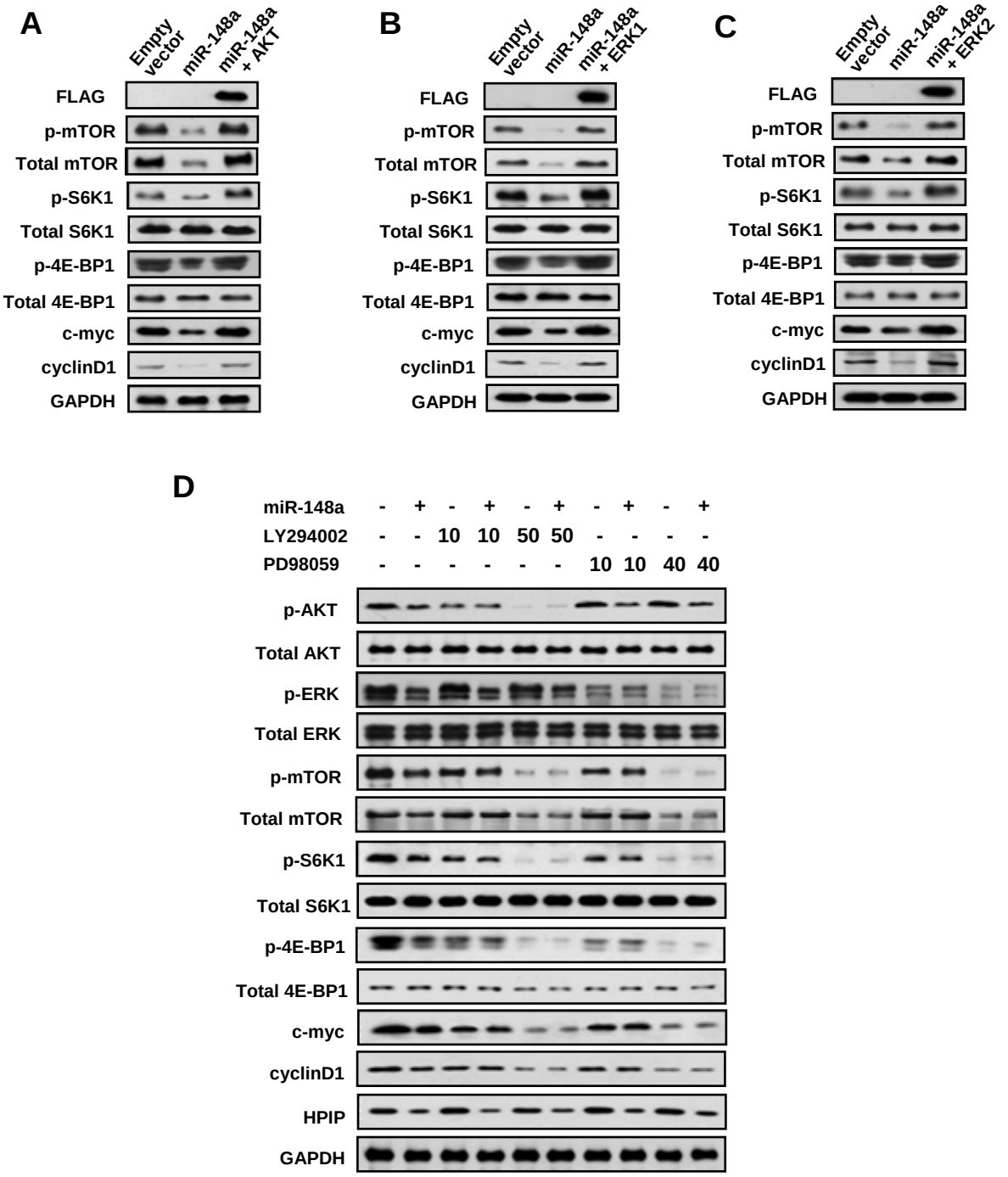
Supplemental Figure 3. HPIP activates AKT and ERK through its interaction with Src kinase and the p85 subunit of PI3K. (A) Cell lysates from HepG2 cells were immunoprecipitated with anti-HPIP or normal IgG, followed by immunoblot with the indicated antibodies. (B and C) Effect of either PI3K inhibitor wortmannin (B) or Src kinase inhibitor PP2 (C) on HPIP-mediated activation of AKT and ERK. HepG2 cells transfected with HPIP were treated with 20 μM wortmannin or 50 μM PP2 for 24 h.

Supplemental Figure S4



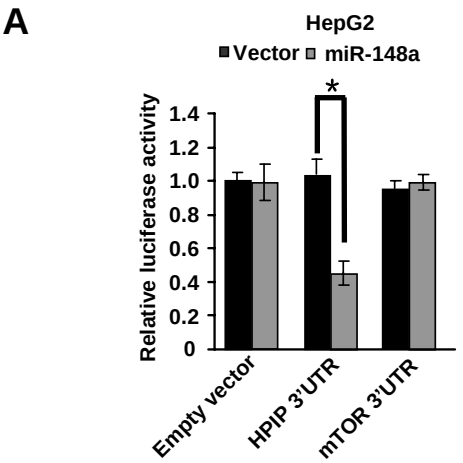
Supplemental Figure 4. HPIP regulates mTOR signaling and miR-148a modulation of mTOR signaling depends on HPIP. Western blot analysis of HepG2 cells transfected with miR-148a, HPIP siRNA, miR-148a plus HPIP siRNA, or miR-148a together with HPIP siRNA1 and siRNA-resistant HPIP (HPIP-R).

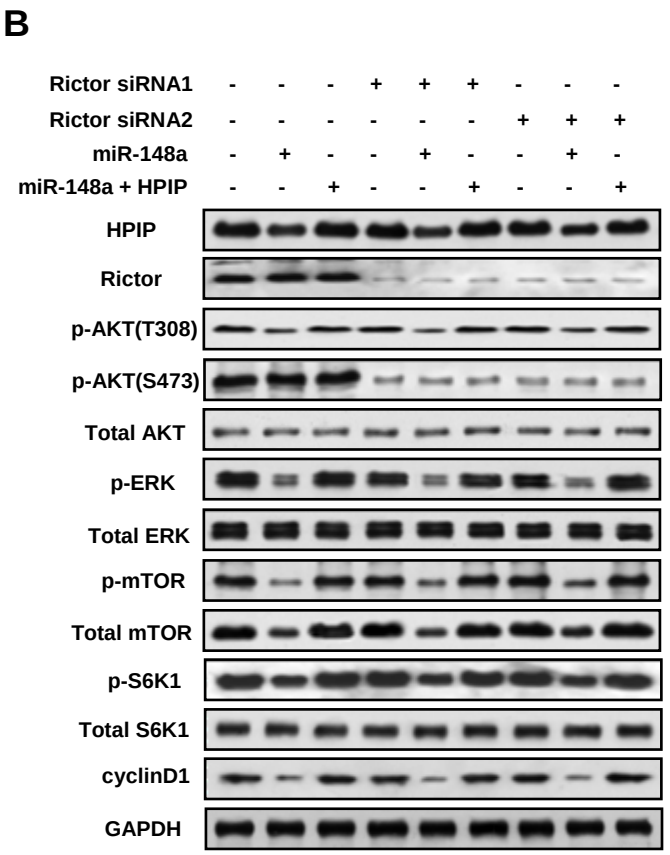
Supplemental Figure S5

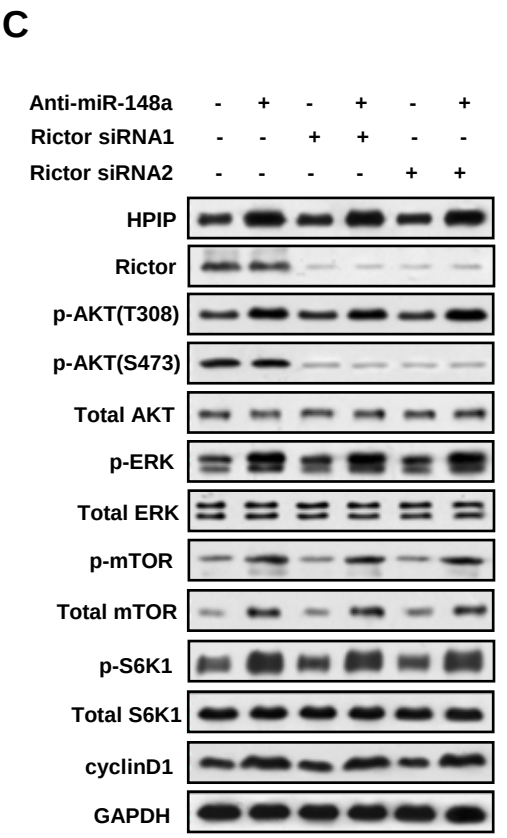


Supplemental Figure 5. MiR-148a regulates mTOR signaling through inhibition of AKT and ERK1/2. (**A-C**) Western blot analysis of HepG2 cells transfected with miR-148a or miR-148a plus AKT, ERK1 or ERK2. (**D**) Western blot analysis of HepG2 cells transfected with miR-148a and treated with LY294002 (10 μM or 50 μM) or PD98059 (10 μM and 40 μM) for 24 h.

Supplemental Figure S6

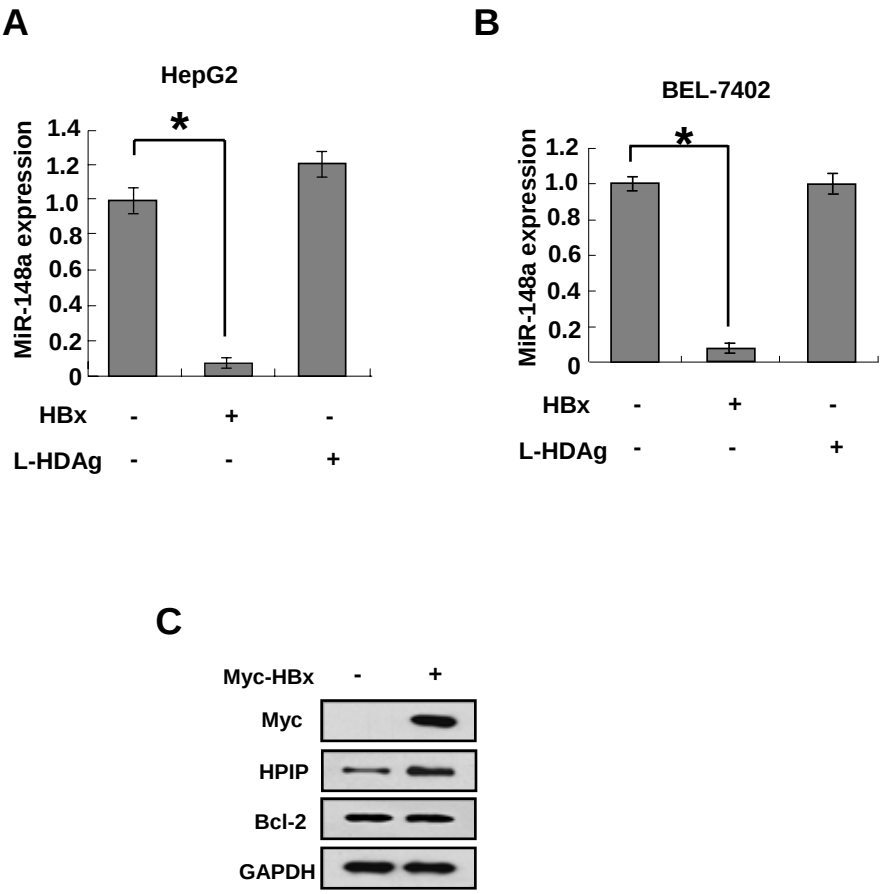






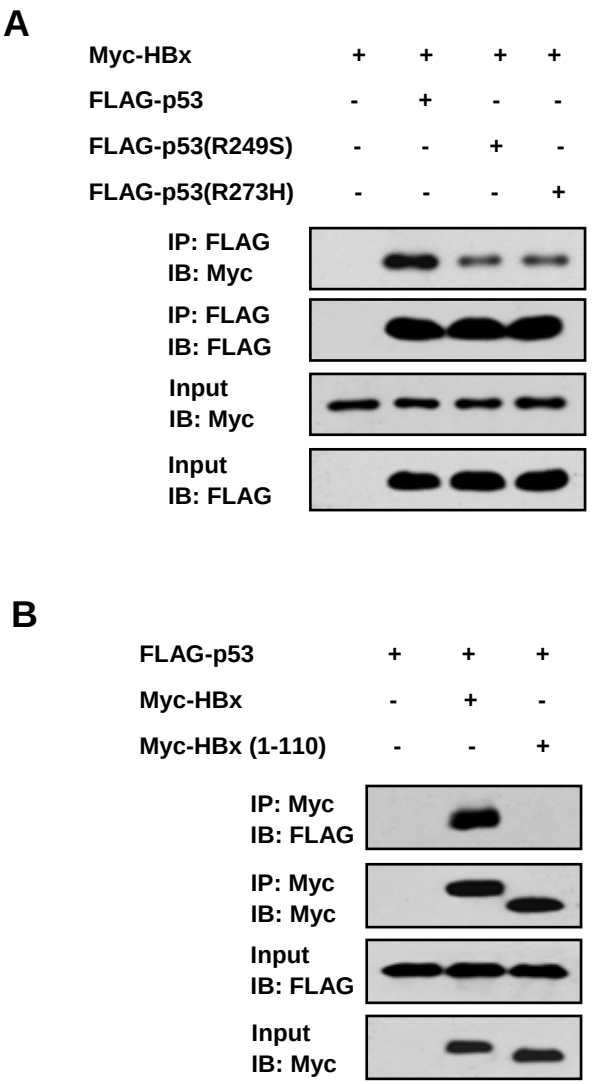
Supplemental Figure 6. Activation of mTORC2 is not required for miR-148a modulation of mTOR signaing. (A) MiRNA luciferase reporter assays in HepG2 cells transfected with miR-148a and HPIP 3'UTR reporter or mTOR 3'UTR reporter. All values shown are mean ± SD of triplicate measurements and have been repeated 3 times with similar results (*p< 0.01). (B) Western blot analysis of HepG2 cells transfected with miR-148a or miR-148a plus HPIP with or without Rictor siRNAs. (C) Western blot analysis of HepG2 cells transfected with anti-miR-148a or anti-miR-148a plus Rictor siRNAs.

Supplemental Figure S7



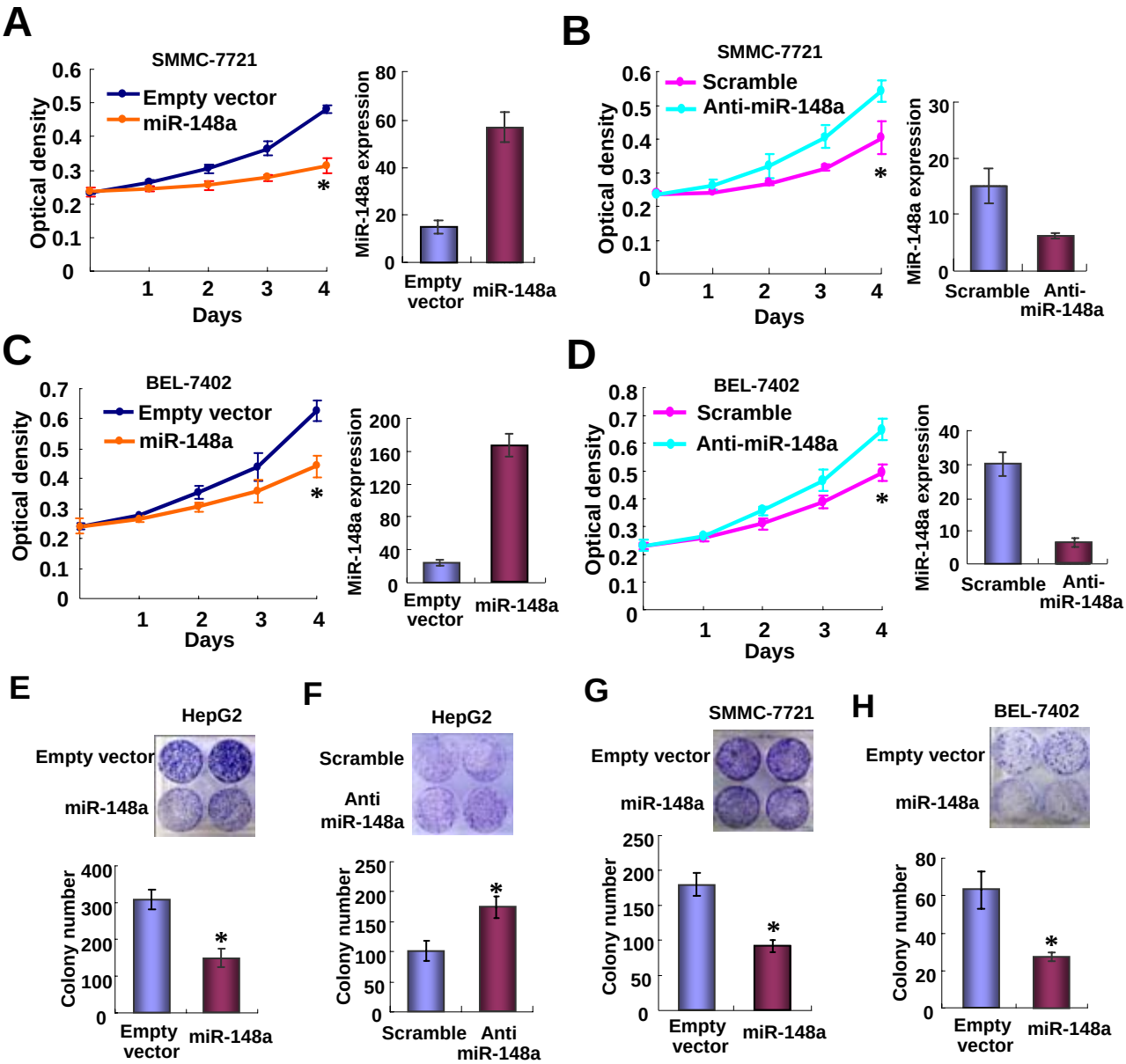
Supplemental Figure 7. HBx reduces miR-148a expression. (**A** and **B**) HepG2 (A) and BEL-7402 (B) cells transfected with HBx or L-HDAg were used for real-time RT-PCR with miR-148a primers (*p < 0.01). (**C**) Immunoblot analysis of HepG2 cells transfected with Myc-tagged HBx or empty vector.

Supplemental Figure S8



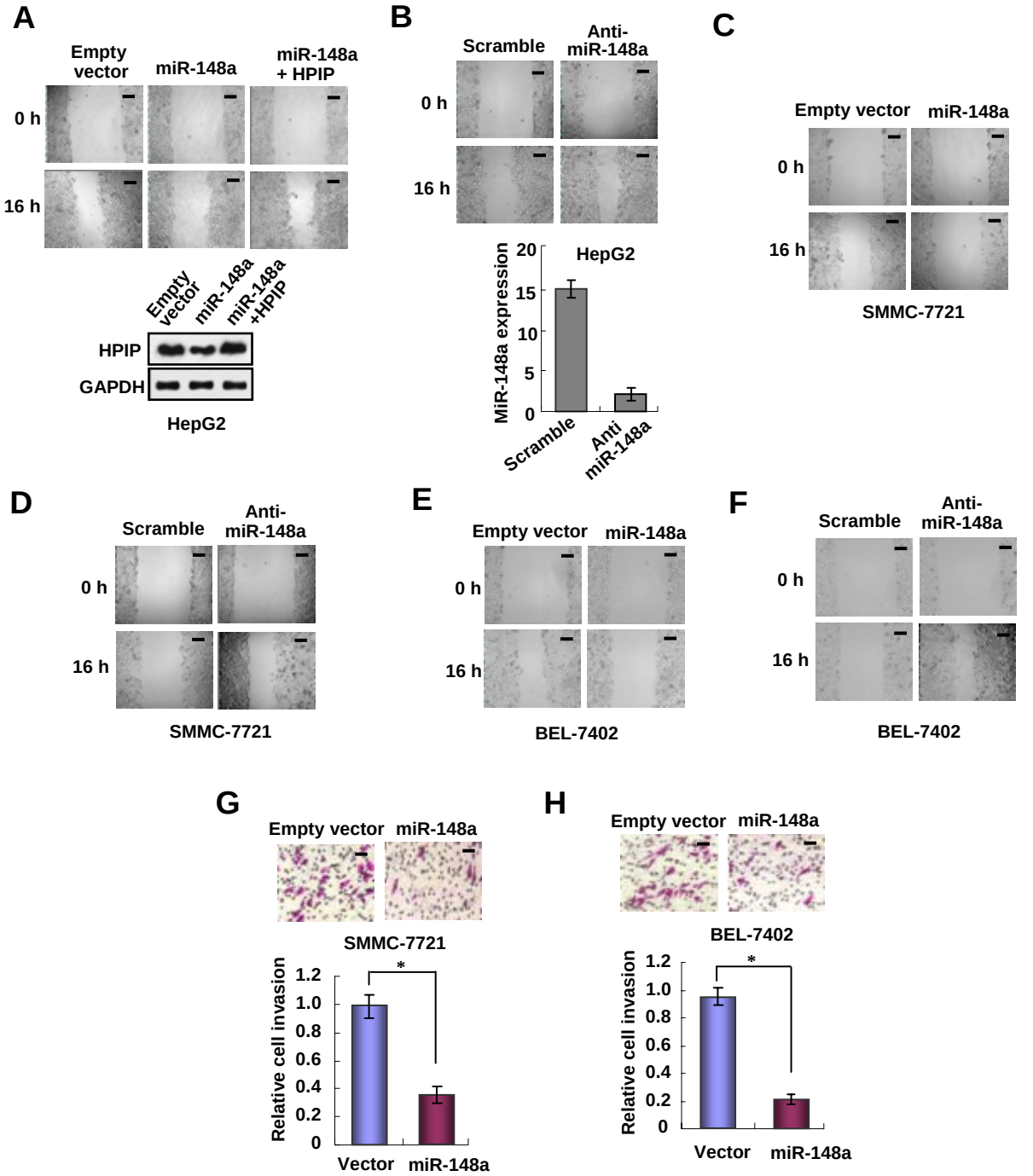
Supplemental Figure 8. Mutation of p53 and deletion of HBx C-terminus alter the interaction between p53 and HBx. (**A**) Myc-tagged HBx and FLAG-tagged p53, p53(R249S) or p53(R273H) were co-transfected into 293T cells. Cell lysates were immunoprecipitated by anti-FLAG antibody, and precipitates were immunoblotted with anti-Myc antibody. (**B**) FLAG-tagged p53 and Myc-tagged HBx or HBx(1-110) were co-transfected into 293T cells. Cell lysates were immunoprecipitated with anti-Myc, and precipitates were immunoblotted with anti-FLAG.

Supplemental Figure S9



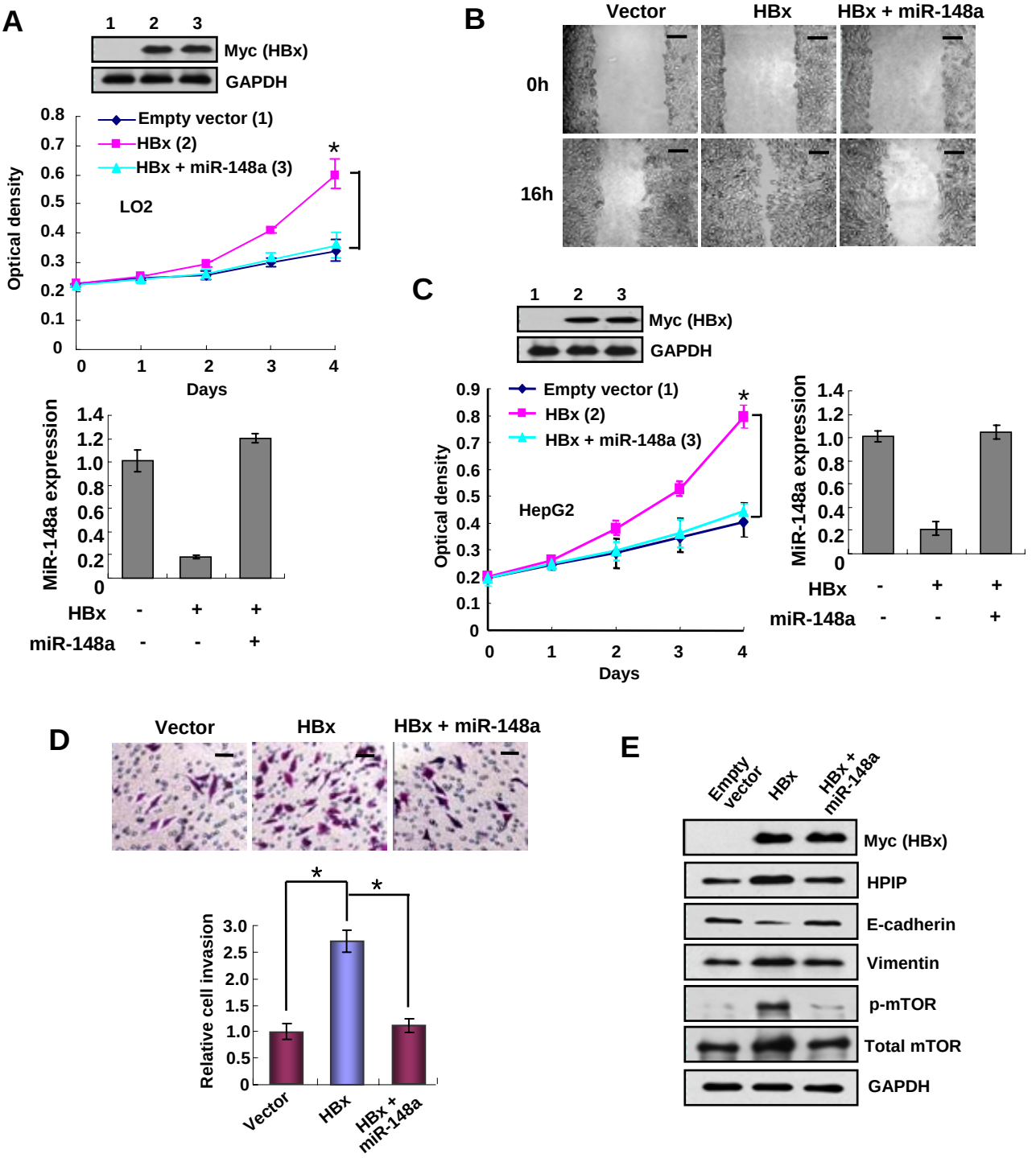
Supplemental Figure 9. MiR148a reduces HCC cell growth. (**A-D**) SMMC-7721 (A and B) and BEL-7402 (C and D) cells expressing miR-148a (A and C) or anti-miR-148a (B and D) were grown in regular medium. At specified times, cell numbers were determined by CCK-8 assay. Representative real-time RT-PCR shows miR-148a expression (a-d, right panel). (**E-H**) Colony formation assays for HepG2 cells expressing miR-148a (E) or anti-miR-148a (F), and SMMC-7721 (G) and BEL-7402 (H) cells expressing miR-148a. All values shown are mean ± SD of triplicate measurements and have been repeated 3 times with similar results (* $p < 0.01$ versus empty vector or scramble vector).

Supplemental Figure S10



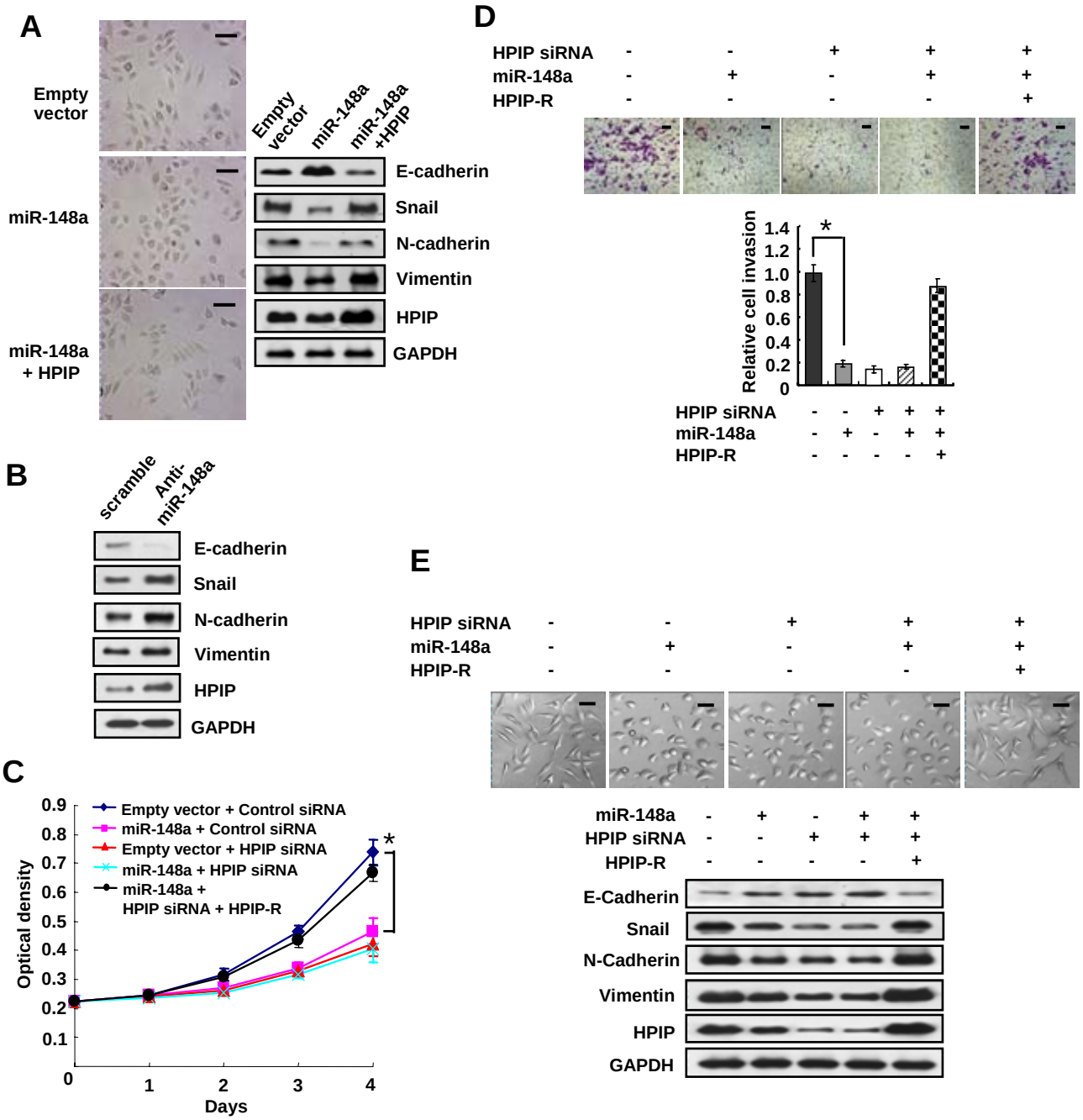
Supplemental Figure 10. MiR-148a suppresses HCC cell migration and invasion. (**A** and **B**) Wound-healing assays were conducted in HepG2 cells expressing miR-148a or miR-148a plus HPIP (A) or anti-miR-148a (B). Cell migration was measured 16 h after cells were scratched. Expression of HPIP and miR-148a were analyzed by Western blot (A) and real-time RT-PCR (B), respectively. Scale bar, 100 μM. (**C-F**) Wound-healing assays were conducted in SMMC-7721 (C and D) and BEL-7402 (E and F) cells expressing miR-148a (C and E) or anti-miR-148a (D and F). (**G** and **H**) Cell invasion was evaluated in SMMC-7721 (G) and BEL-7402 (H) cells expressing miR-148a using a Matrigel invasion chamber. Invasive cells were fixed and stained with crystal violet (upper panels). Scale bar, 100 μM. All values shown are mean ± SD of triplicate measurements and have been repeated 3 times with similar results (*$p < 0.01$).

Supplemental Figure S11



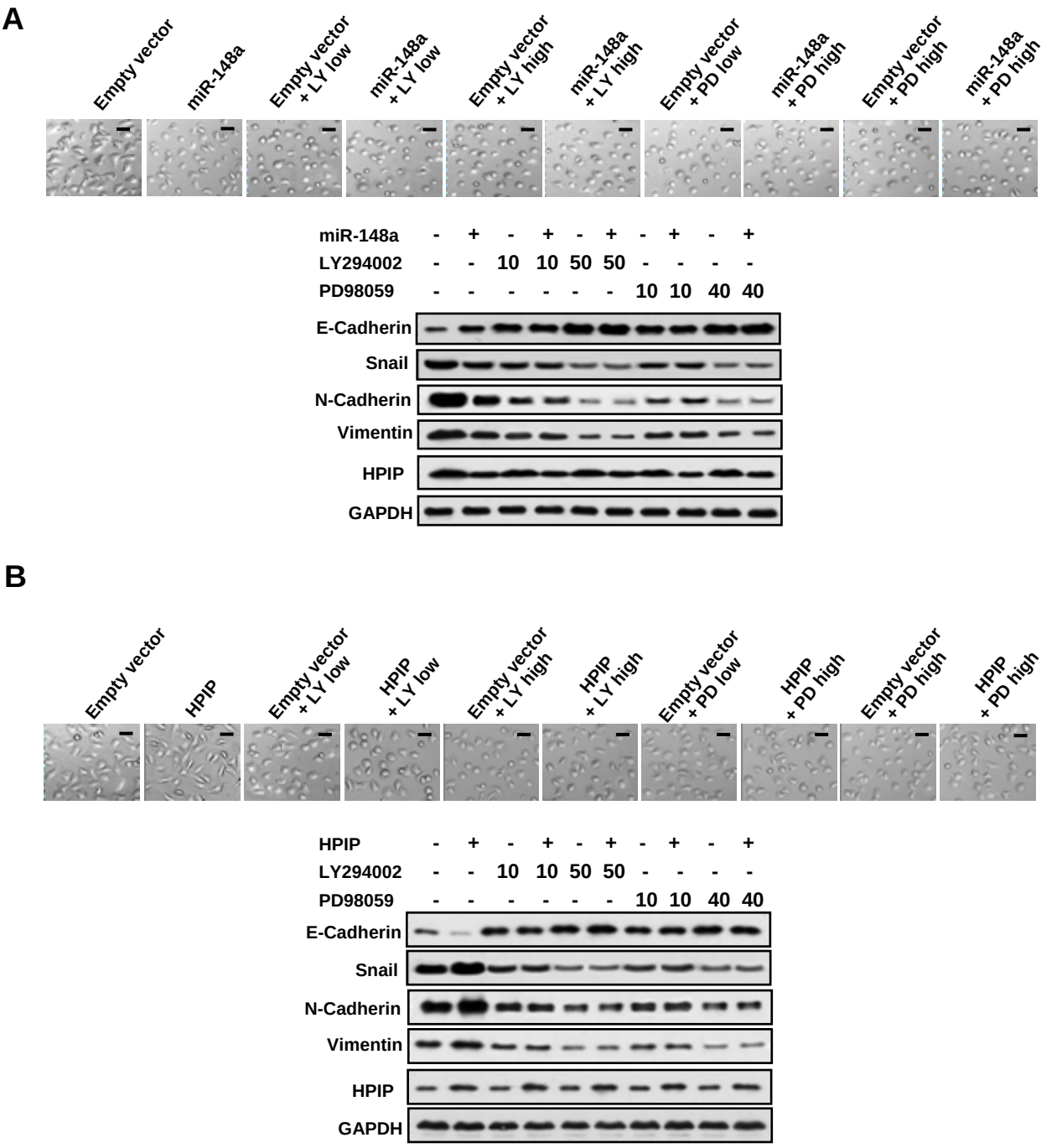
Supplemental Figure 11. HBx enhances liver cell growth and migration through inhibition of miR-148a. (**A**) LO2 cells transfected with Myc-tagged HBx or Myc-tagged HBx puls miR-148a were grown in regular medium. At specified times, cell numbers were determined by CCK-8 assay. The representative immunoblot with anti-Myc shows Myc-HBx expression. Representative real-time PCR shows miR-148a expression (lower panel) (*p < 0.01). (**B**) Wound-healing assays were performed in LO2 cells transfected with HBx or HBx puls miR-148a. Cell migration was measured 16 h after cells were scratched. Scale bar, 100 μm. (**C**) HepG2 cells were transfected and analyzed as in (A). (**D**) Cell migration was evaluated in HepG2 cells transfected as in (C) using a migration chamber. Scale bar, 100 μM. All values shown are mean ± SD of triplicate measurements (*p < 0.01). (**E**) Western blot analysis of LO2 cells transfected with Myc-tagged HBx or Myc-tagged HBx plus miR-148a.

Supplemental Figure S12



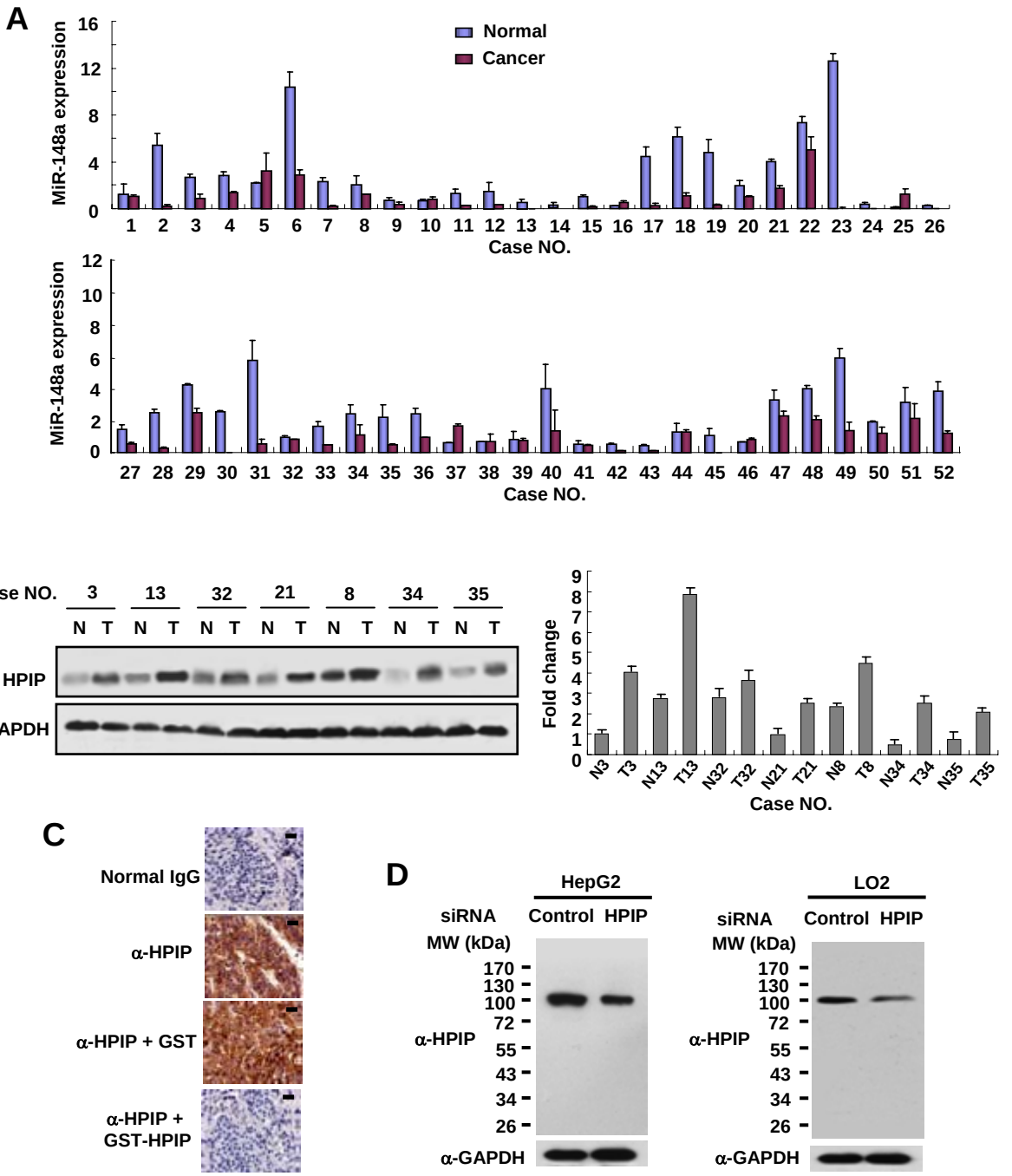
Supplemental Figure 12. MiR-148a represses cell proliferation, invasion and EMT through inhibition of HPIP expression. (**A** and **B**) Immunoblot analysis of HepG2 cells transfected with miR-148a or miR-148a plus HPIP (**A**) or anti-miR-148a (**B**). Morphologic changes are shown in the photographs (A, left panel). Scale bar, 100 μm. (**C**) HepG2 cells expressing miR-148a, HPIP siRNA1, miR-148a plus HPIP siRNA1, or miR-148a together with HPIP siRNA1 and siRNA-resistant HPIP (HPIP-R) were cultured in regular medium. At specified times, cell numbers were determined by CCK-8 assay. (**D**) Cell invasion was evaluated in HepG2 cells expressing miR-148a, HPIP siRNA1, miR-148a plus HPIP siRNA1, or miR-148a together with HPIP siRNA1 and HPIP-R using a Matrigel invasion chamber. (**E**) HepG2cells were transfected with miR-148a, HPIP siRNA1, miR-148a plus HPIP siRNA1, or miR-148a together with HPIP siRNA1 and HPIP-R, and were analyzed as in (A). All values shown are mean ± SD of triplicate measurements and have been repeated 3 times with similar results (*$p < 0.01$)

Supplemental Figure S13



Supplemental Figure 13. MiR-148a/HPIP regulates EMT through modulation of AKT and ERK1/2. HepG2 cells were transfected with miR-148a (A) or HPIP (B) and treated for 24 h with 10 μM LY294002 (LY low), 50 μM LY294002 (LY high), 10 μM PD98059 (PD low), or 40 μM PD98059 (PD high). Western blot was performed with the indicated antibodies. Morphologic changes are shown in the photographs (upper panel). Scale bar, 100 μm.

Supplemental Figure S14



Supplemental Figure 14. MiR-148a and HPIP expression in HCC patients. (**A**) Real-time RT-PCR analysis of miR-148a expression in 52 pairs of human cancerous liver tissues and adjacent normal liver tissues. (**B**) Representative immunoblots of HPIP in 7 pairs of human liver tumors (T) and adjacent normal liver tissues (N). The densitometric quantitation of HPIP bands normalized to GAPDH from 3 independent experiments is shown (right panel) (mean ± SD). (**C**) Immunohistochemical staining of liver cancer specimens incubated with normal IgG or anti-HPIP. To validate antibody specificity, the anti-HPIP was pre-incubated with recombinant GST-HPIP protein or GST for 1 h prior to applying to tissue. Original magnification, ×20. Scale bar, 100 μm. (**D**) Immunoblot analysis of lysates from HepG2 (left panel) or LO2 (right panel) cells transfected with control siRNA or HPIP siRNA using antibodies specific for anti-HPIP. MW, molecular weight.