SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. HGF inhibits trametinib-induced apoptosis in UM cells. (A) UM001 cells were treated with DMSO, 10 ng/ml HGF, 50 nM trametinib, a combination of HGF and trametinib, respectively, for 48 hours. Cells were then subjected to Annexin V/PI staining. Representative traces are shown.

Supplemental Figure 2. Bim-EL or Bmf expression renders UM cells susceptible to apoptosis. (A) UM001 cells were infected with adenovirus harboring indicated cDNAs for 16 hours. Cells were then lysed and cell lysates were probed with Bim-EL, Bmf, GFP and actin antibodies. (B) UM001 cells were infected with the indicated adenoviruses for 24 hours. Cells were then stained with Annexin V to determine apoptosis. The average percentage of Annexin V positive cells is shown from three independent experiments. ***P<0.001, based on two-tail Student's t-test assuming unequal variance.

Supplemental Figure 3. Human hepatic stellate cell (HHSteC) conditioned medium activates HGF-cMET signaling. (A) Early passage HHSteC do not express fibroblast markers. Cell lysates from UM001, UM004, CAF41 (cancer-associated fibroblast 41), CAF43 (cancer-associated fibroblast 43), passage 2 (p2) HHSteC, p3 HHSteC, p5 HHSteC and p6 HHSteC were stained with α-SMA, FAP and actin antibodies. (B) UM001 and UM004 cells were incubated with unconditioned medium or passage 2 HHSteC conditioned medium for 1 hour or 48 hours. Protein cell lysates were subjected to RPPA analysis. Supervised clustering of proteins that were regulated by stellate cells conditioned medium is shown. (C) Levels of HGF in passage 2, passage 5 and passage 6 HHSteC conditioned medium were determined by ELISA. (D) 92.1 cells were cultured in LX-2 conditioned medium or treated with 10 ng/ml HGF for 1 hour. Cell lysates were probed with phospho cMET, cMET and actin antibodies (bottom panel).

Supplemental Figure 4. Human hepatic stellate cell (HHSteC) conditioned medium effects on migration and invasion of UM cells. UM001 and UM004 cells were cultured overnight in serum-free medium. The next day, 1 x 10^4 cells were placed inside 8.0 µm pore-size cell culture inserts. For invasion assay, the inserts were first coated with 0.75 mg/ml Matrigel for 1 hour before plating cells inside each chamber. Cells were allowed to migrate or invade for 16 hours towards an attractant of stellate cell culture medium or conditioned medium collected from passage 3 stellate cells. Chamber filters were fixed in buffered formalin and stained with crystal violet. The cells in the inner chamber were removed. Images were taken with a Nikon TiEclipse inverted microscope and representative microscopic images of the cells at 100x magnification are shown.

Supplemental Figure 5. Isoform specific PI3K inhibitors differentially block HGF-mediated activation of AKT in UM001 cells. UM001 cells were pretreated with increasing doses of GDC0032, TGX221, BYL719 and IPI145 for 6 hours. Cells were then stimulated with 10 ng/ml HGF for 30 minutes. Cell lysates were probed with pAKT, AKT PI3Kα, PI3Kβ, PI3Kγ, PI3Kδ and actin antibodies. (B) UM001 cells were treated 50 nM trametinib, 10 ng/ml HGF, 0.5 µM of GDC0032, TGX221, BYL719 or IPI145 respectively or in combination for 3 days. Cell growth was determined by crystal violet staining. Representative microscopic images are shown (100x magnification). Scale bar is equal to 100 µm.