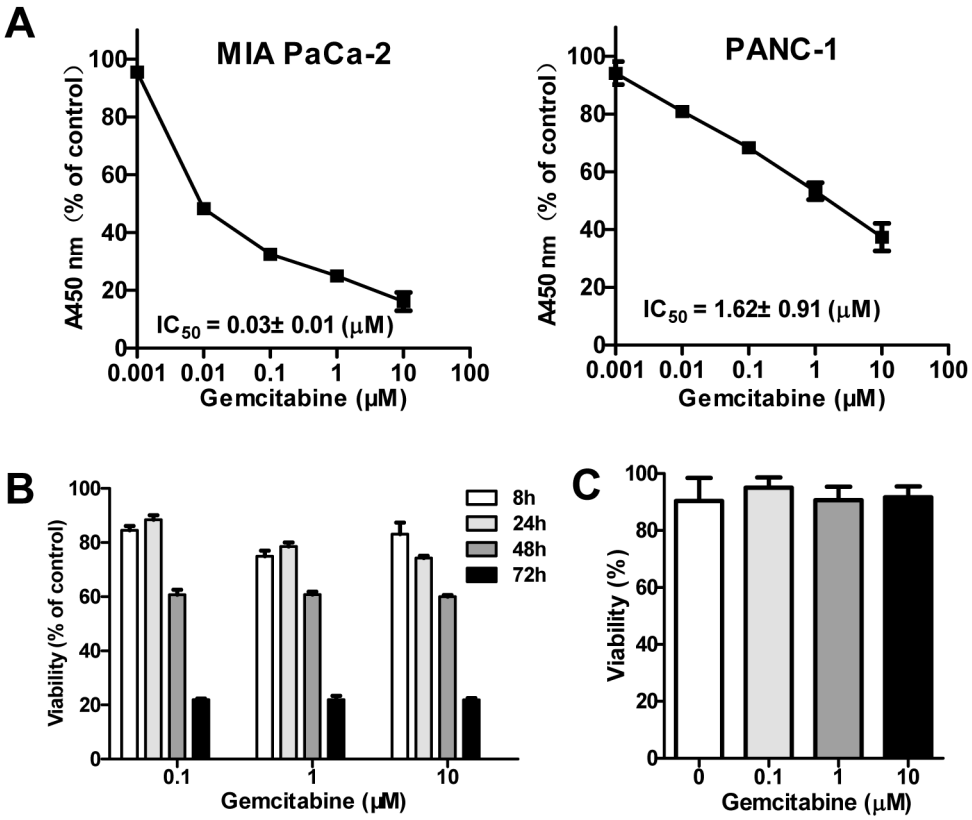
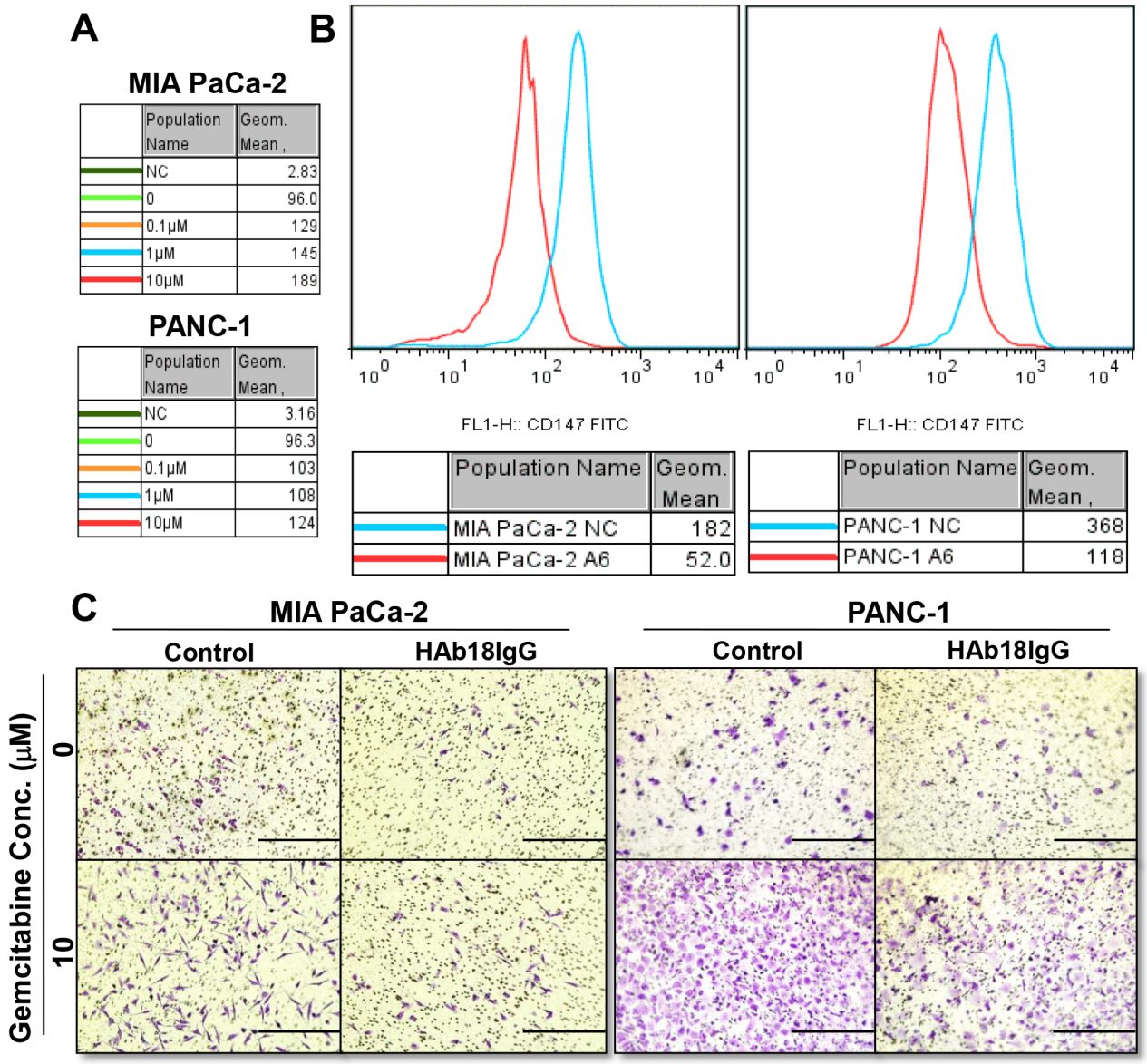
Gemcitabine enhances cell invasion via activating HAb18G/CD147-EGFR-pSTAT3 signaling

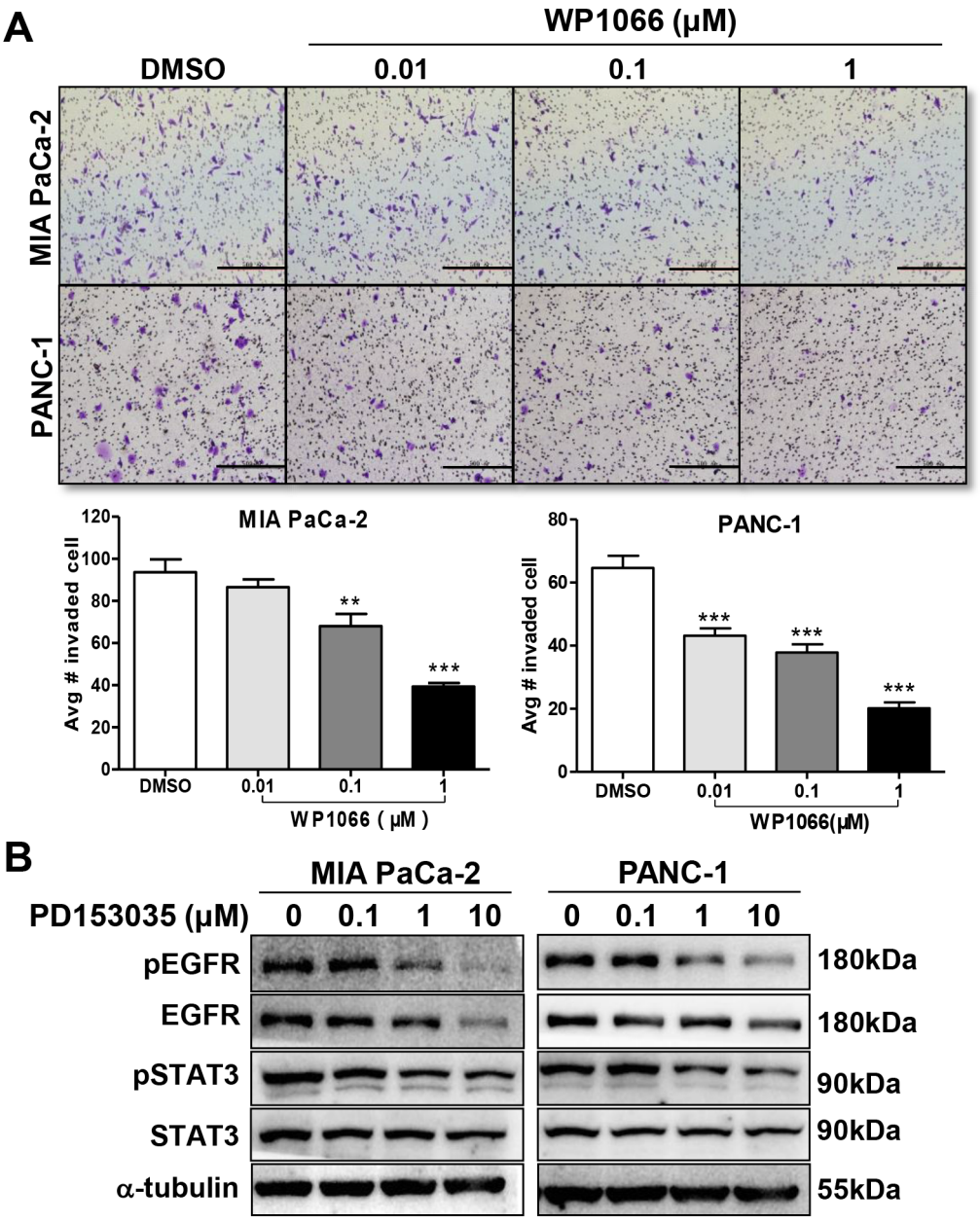
SUPPLEMENTARY FIGURES



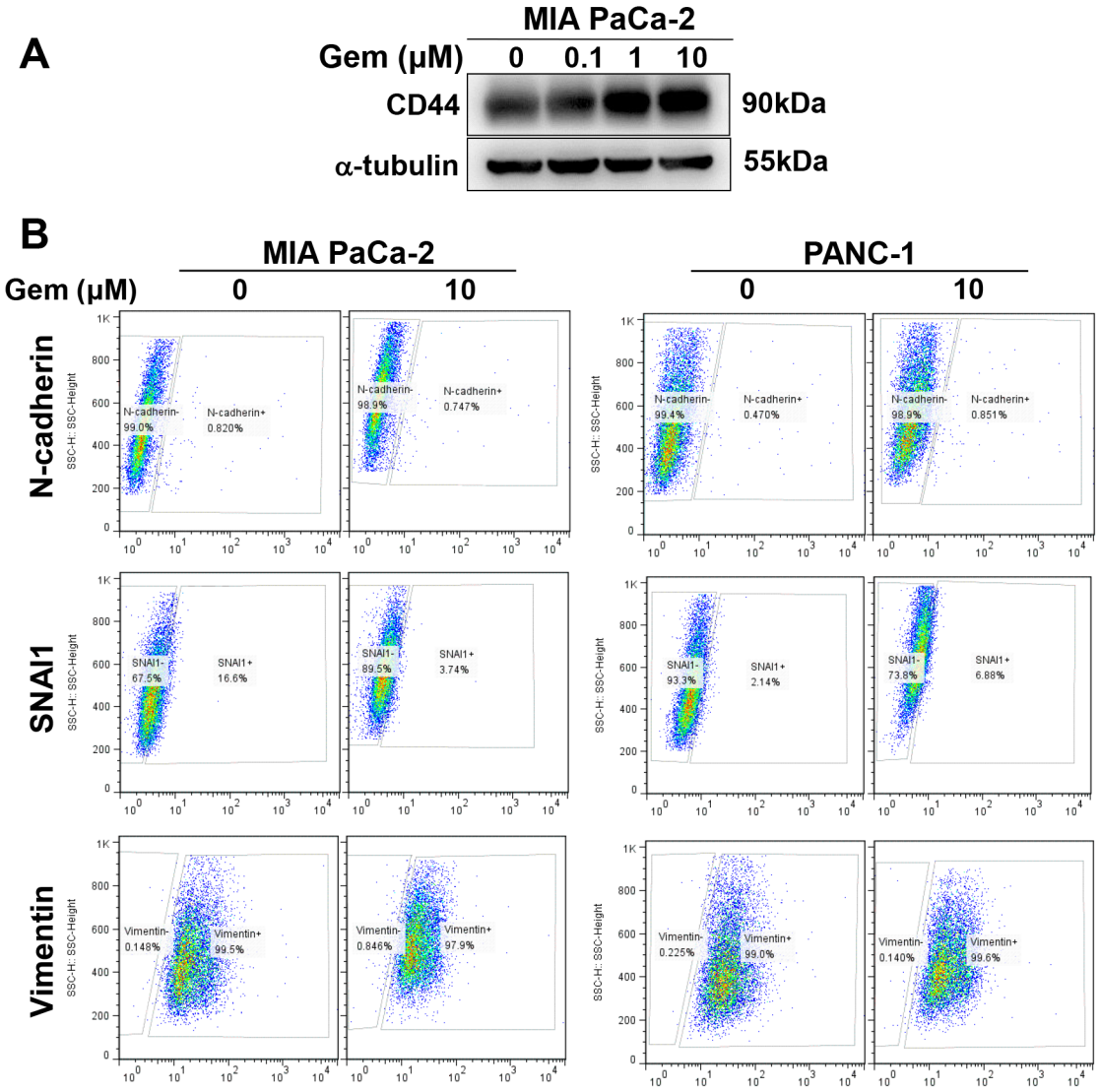
Supplementary Figure S1: Gemcitabine enhances the migration and invasion of pancreatic cancer cells. A. Chemosensitivity of MIA PaCa-2 cells and PANC-1 cells to gemcitabine. IC_{50} values were calculated at 72 hours by fitting sigmoidal dose-response curve using GraphPad Prism version 5.0. **B.** Relative cell viability after treatment with various concentrations of gemcitabine (0, 0.1, 1, or 10 μM) for different time points (8 to 72 hours) was examined by WST-8 assay. MIA PaCa-2 cells treated with PBS were used as control. **C.** Cell viability measurement by hemocytometer. MIA PaCa-2 cells were treated with gemcitabine for 24 hours at various concentrations (0, 0.1, 1, or 10 μM) and the cell viability was examined immediately.



Supplementary Figure S2: HAb18G/CD147 is required for gemcitabine-enhanced invasion of pancreatic cancer cells. Cells were labeled with FITC-conjugated anti-human CD147 antibody, and isotype-matched mouse immunoglobulin was used as a control. **A.** Flow cytometry analysis of the HAb18G/CD147 membrane levels in pancreatic cancer cells treated with different doses of gemcitabine (0, 0.1, 1, or 10 μM) for 24 hours. **B.** Flow cytometry analysis of the membrane levels of HAb18G/CD147 in CD147 knock-down cells. NC, non-target shRNA control; A6, CD147 shRNA. **C.** *In vitro* invasion assay of pancreatic cancer cells treated with gemcitabine (10 μM, 24 hours) alone or in combination with 30 μg/ml HAb18IgG. Cells that migrated/invaded to the lower surface of the filter were fixed, stained, imaged, and counted in 10 random view fields. Bars = 500 μm as indicated. $^{**}P < 0.01$; $^{***}P < 0.001$ compared with control.



Supplementary Figure S3: EGFR-STAT3 signaling is involved in gemcitabine-enhanced invasion. A. *In vitro* invasion of pancreatic cancer cells treated with different doses of WP1066 (0, 0.01, 0.1, or 1 μM) for 24 hours. **B.** Western blot of pSTAT3, STAT3, pEGFR, and EGFR expression in pancreatic cancer cells treated with different doses of PD153035 (0, 0.1, 1, or 10 μM) for 24 hours.



Supplementary Figure S4: CD44 and EMT markers expression upon gemcitabine treatment. A. Western blot of CD44 expression in pancreatic cancer cells treated with different doses of gemcitabine (0, 0.1, 1, or 10 μM) for 24 hours. **B.** Flow cytometry analysis of the EMT markers' (N-cadherin, SNAI1 and Vimentin) membrane levels in pancreatic cancer cells treated with gemcitabine (10 μM, 24 hours).