Supplementary Materials and Methods

RT-PCR. Total RNA extraction from tumor cells was performed with Trizol Reagent (Invitrogen). Then, 2 μg of total RNA was reverse-transcribed with the First Strand cDNA Synthesis Kit (Promega) to synthesize cDNA samples. Subsequently, 2μl of cDNA product was then subjected to PCR amplification with Taq DNA polymerase (Qiagen, Valencia, CA) on a thermal cycler using the following primers. The PCR primers used to detect each factor were as follows: FoxM1, sense strand 5'-ACGTCCCCAAGCCAGGCTC-3', antisense strand 5'-CTACTGTAGCTCAGGAATAA-3'; Cav-1, sense strand 5'-AATACTGGTTTTACCGCTTGCT-3', antisense strand 5'-CATGGTACAACTGCCCAGATG-3'; GAPDH, sense strand 5'-CACCATTGGCAATGAGCGGTTC-3', antisense strand 5'-AGGTCTTTGCGGATGTCCACGT-3'. The PCR products were loaded onto 2% agarose gels and visualized with ethidium bromide under UV light.

Chromatin immunoprecipitation assay. Tumor cells (2×10^6) were prepared for chromatin immunoprecipitation (ChIP) assay with the ChIP assay kit (Millipore Technology, Billerica, MA) according to the manufacturer's protocol. The resulting precipitated DNA samples were analyzed using PCR to amplify a 345-bp region of the Cav-1 promoter with the primers 5'-TCCAGCTTGGGTGACAGAGGAC-3' (sense) and 5'- CGAATCCCTGTGACACAAAA-3' (antisense); a 108-bp region of the Cav-1 promoter with the primers 5'-TGGGAAAATCCAACTTTTGAA-3' (sense) and 5'- AGAATGGCAGGGAAAGTGTCAC-3' (antisense); a 181-bp region of the Cav-1 promoter with the primers 5'-GTGACACTTTCCCTGCCATTCTT-3' (sense) and 5'-TGTGGGTTGGGAGTAAGAGG-3' (antisense). The PCR products were resolved electrophoretically on a 2% agarose gel and visualized using ethidium bromide staining.

Cell scratch-wound assay. Pancreatic cancer cells were grown in 6-well plates until confluence. A wound was generated by scraping with a 10 μl tip. After 12 hr, the cells in the

wounded monolayer were photographed and cell migration was assessed by measuring gap sizes at multiple fields.

Cell migration assay. Cell migration assays was conducted using a modified 24-well Boyden chamber with a membrane that was uncoated with Matrigel (BD Biosciences). Ten percent fetal bovine serum-containing medium was placed in the lower chambers to be used as a chemoattractant. Pancreatic cancer cells (3×10^5) in a 300-μL volume of serum-free medium were placed in the upper chambers and incubated at 37°C for 24 h. Migrated cells on the bottom surface of the filter were fixed, stained with H&E, and counted under a microscope in five randomly selected fields at a magnification of 200×.

Cell invasion assay. Invasion assay was performed using a specialized Chemicon invasion chamber which included a 24-well tissue culture plate with 12 cell culture inserts (Millipore). The inserts contained an 8 μm pore size polycarbonate membrane with a precoated thin layer of basement membrane matrix (ECMatrix). Ten percent fetal bovine serum-containing medium was placed in the lower chambers to act as a chemoattractant. Pancreatic cancer cells (3×10^5) in a 300-μL volume of serum-free medium were placed in the upper chambers and incubated at 37°C for 48 h. Invasive cells on the lower surface of the membrane, which had invaded the ECMatrix and had migrated through the polycarbonate membrane, were stained by the staining solution, and counted under a microscope in five randomly selected fields at a magnification of 200×.

Cell immunofluorescence. COLO357, L3.7, Panc02 and Panc02-H7 cells were cultured on Falcon chamber slides (BD Biosciences) at up to 50-60% confluence before being fixed with 4% paraformaldehyde and permeabilized with 0.3% Triton X-100. The cells were then immersed three times in phosphate-buffered saline, incubated with indicated Cav-1 primary antibody (BD Biosciences) overnight at 4°C and corresponding Alexa Fluor-conjugated secondary antibodies (Invitrogen) for 1 h at room temperature, and mounted using a mounting medium containing 4',6-

diamidino-2-phenylindole. Microscopic images of cells were obtained using an Axio Observer A microscope (Zeiss).

Animals. Pathogen-free female athymic BALB/c nude mice were purchased from the National Cancer Institute. The animals were maintained in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International in accordance with the current regulations and standards of the U.S. Department of Agriculture and Department of Health and Human Services.

Subcutaneous tumor growth. Tumor cells (1×10^5) in 0.1 mL of Hank's balanced salt solution were injected subcutaneously into the right scapular region of nude mice. The tumor-bearing mice were sacrificed when they became moribund or on day 35 after inoculation and the tumors were removed and weighed.

Experimental Liver metastases. 1×10^5 L3.7 or 1×10^6 COLO357 cells were injected intravenously via the ileocolic vein. The mice were killed day 21 after tumor-cell inoculation or when they appeared to be moribund. Their livers were then removed, and surface metastases were counted after dividing the liver into its individual lobes. Every surface was examined by two investigators who were unaware of the experimental protocol and scored separately (33). Tissue was then placed in 10% buffered formalin, immersed in an ascending series of alcohols, and paraffin embedded. Sections were cut and stained with hematoxylin and eosin.