Supplementary Figure Legends

Supplementary Fig. S1. FOXM1 overexpression and its direct association with clinicopathological parameters in pancreatic cancer cases. Staining for FOXM1 protein was performed using a TMA with 70 primary pancreatic tumors. Increased FOXM1 expression correlated with decreased tumor differentiation and increased tumor grade (grades 1 and 3) and positively correlated with disease stage (stages 1 and 4). FOXM1 expression in primary pancreatic tumor specimens obtained from patients with lymph node or distant metastasis was significantly higher than that in primary tumor specimens obtained from patients without metastasis.

Supplementary Fig. S2. FoxM1 isoforms and PCR primers. A, Specific primers for FoxM1 isoforms. B, PCR primer locations of FoxM1a, 1b, and 1c isoforms. C, The FoxM1abc primers amplified predicted sizes of isoforms FoxM1a, 1b, and 1c when their respective plasmids were used, whereas only isoform FoxM1c was amplified when FoxM1 cDNA was used (the cDNA contained different levels of isoform FoxM1a, 1b, and 1c). D, specific primers for FoxM1a, 1b, and 1c amplified predicted sizes of FoxM1a, 1b, and 1c when their respective plasmids were used as templates. E, Relative expression levels of FoxM1a, 1b, and 1c in non-metastatic COLO357 and metastatic L3.7 cells as determined by using FoxM1a, 1b, and 1c isoform-specific primers.

Supplementary Fig. S3. FOXM1b, FOXM1b1, and FOXM1b2 expression in pancreatic cancer cells. A, schematics of the structures of five alternative splice FOXM1 isoforms. Note: FOXM1b1 had a GCA deletion (amino acids 168) and CAG insertion (amino acids 327), whereas FOXM1b2 had only the GCA deletion. B, from isoform FOXM1b, we further determined the proportions of FOXM1b, FOXM1b1, and FOXM1b2. qPCR analysis revealed only information on “total” FOXM1b isoforms, not information on FOXM1b1 or FOXM1b2. We used the RNAs
collected as described above, converted them to cDNAs, subcloned them into TA-cloning vectors, and collected multiple clones of each cDNA sample for sequencing using unique primers (Supplementary Fig. S2A) to distinguish FOXM1b, FOXM1b1, and FOXM1b2 and measure the relative abundance of each of them. C, using a promoter reporter assay, we found that FOXM1b, FOXM1b1, and FOXM1b2 transactivated their downstream genes, including VEGF and MMP-2, suggesting that these isoforms have very similar functions. The VEGF and MMP-2 promoters used in this experiment were described previously (13,14).

**Supplementary Fig. S4.** Influence of altered expression of FOXM1 isoforms on pancreatic tumor growth and metastasis. A, Gross photos of mice without (A1) or with BxPC-3 liver metastases (A2, red arrowheads), and a representative liver with metastases (A3). B, BxPC-3 liver sections without (B1) or with metastases (B2, red arrows), and a representative photo of liver metastasis histology (NL, normal liver; HM, hepatic metastasis). C, Experimental liver metastases. BxPC-3 (C1 & C2) and PANC-1 cells (C3 & C4) with increased (by transfection) or decreased (by knockdown) expression of FOXM1a, FOXM1b, or FOXM1c were intravenously injected via the ileocolic vein into groups of 10 mice. Experimental hepatic metastases were determined 35 days after tumor cell injections.

**Supplementary Fig. S5.** Suppression of pancreatic cancer cell migration and invasion by knockdown of FOXM1 expression. Untransfected L3.7 cells (control) and L3.7 cells transfected with control siRNA (mock) or si-FOXM1 were used. A, cell scratch-wound assay. B, cell migration assay. C, cell invasion assay. Assay details are provided in Supplementary Materials and Methods. Note: inhibition of FOXM1 expression substantially inhibited the horizontal and vertical migration and invasion of tumor cells. *P < 0.01 (Student t-test).
Supplementary Fig. S6. FOXM1 promoter activity in pancreatic cancer cells. A, schematic of the structure of the full-length FOXM1 promoter pFXM1-2469 and its deletion mutants. B, the FOXM1 promoter reporters were transfected into six pancreatic cancer cell lines in triplicate. The relative FOXM1 promoter activity in the cell lines was measured 24 hours after transfection.

Supplementary Fig. S7. FOXM1c signaling in pancreatic cancer. During pancreatic cancer development and progression, activation of oncogenes, e.g., K-ras and/or inactivation of tumor suppressors, e.g., TP53 could lead to an increased expression of Sp1 and/or decreased expression of KLF4, Sp1 is a positive regulator of FOXM1 expression, while KLF4 antagonizes Sp1; and KLF4 is also a negative regulator of Sp1 expression. Therefore, a combination of lost expression of KLF4 and overexpression of Sp1 causes overexpression and function of FOXM1c. Subsequently, FoxM1c regulates the expression of a panel of genes, which are key to many aspects of cancer biology, including cell proliferation, EMT, migration/invasion, tumorigenesis, and apoptosis resistance.