Supplemental Methods

Cell Culture

We recently generated an inducible Kras model of pancreatic cancer, the iKras*p53* mouse, to address whether oncogenic Kras is required for maintenance of pancreatic tumors and metastases (14). The iKras*p53* mice express pancreas-specific p48-Cre (Ptf1a-Cre)(Kawaguchi et al. Nat Genet 2002) that activates expression of the transcriptional activator rtTa (from the Rosa26 locus)(Belteki et al. Nucleic Acids Res 2005) and Tp53R172H (p53*)(Olive et al., Cell 2004). rtTa is transcriptionally active in the presence of doxycycline (doxy), thus activating expression of $Kras^{G12D}$ (Kras*)(Fisher et al. Genes Dev 2001). Therefore, p53* is continuously expressed upon Cre recombination, while Kras* expression is reversible. Primary pancreatic cancer cell lines were generated from iKras*p53* tumors. As a feature of this mouse model, pancreatic epithelial cells express EGFP (15), thus allowing their isolation from other cellular components of the tumors. In this study we used two iKras*p53* cell lines with epithelial morphology (4668E and 9805) and two lines with mesenchymal morphology (4668M and 4292). Cells were cultured in RPMI1640 (Gibco) medium containing 10% FBS (Sigma), 1% Antibiotic-Antimycotic reagent (Gibco) and 1 ug/ml of doxycycline (Sigma), and incubated at 37 ^{o}C and 5% CO_2 incubator. The cells were always maintained in doxycycline unless otherwise stated.

Western-blot analysis

Cells were lysed in RIPA buffer (Sigma-Aldrich, R0278) and protease inhibitor (Sigma-Aldrich, P8340). Equal amounts of protein were electrophoresed in 7.5% SDS-PAGE gels (Bio-Rad), and transferred to nitrocellulose membrane (Invitrogen). Membranes were blocked with 5% milk, and primary antibody incubations with rat anti-mouse CD44 antibody (KM114 from BD bioscience in 1:1000 dilution), rabbit anti mouse β-Catenin (Cell Signaling 9587, 1:1000), rabbit anti mouse phospho-GSK-3α/β (Cell Signaling 9331, 1:1000) and mouse anti α-Tubulin (Cell Signaling 3873, 1:1000) were performed overnight at 4°C. Secondary antibodies HRP-conjugated (1:5000) were used and detected with Western Lightning Plus-ECL (Perkin Elmer). Protein bands were visualized on Kodak Biomax XAR film.

Quantitative RT-PCR

Cells for RNA extraction were rinsed with PBS, then isolated using RNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions. Reverse transcription reactions were conducted using a SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). Samples for qRT-PCR were prepared with 1× SYBR Green PCR Master Mix (Applied Biosystems) and various primers (sequences in Supplemental Table 1). All primers were optimized for amplification under reaction conditions as follows: 95°C 2 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Melt curve analysis was performed for all samples after completion of the amplification protocol. *Gapdh* was used as the housekeeping gene expression control.

Short Interfering RNA Transfection

A pool of short interfering RNA duplexes were synthesized and purified by Sigma (St. Lious, MO). Transfection of siRNA was performed using 20-nM CD44 siRNA, ZEB1 siRNA, MT1-MMP siRNA or scrambled control siRNA and Lipofectamine RNAi MAX kit (Invitrogen) according to the manufacturer's instructions. Knock-down level of target genes was determined using qRT-PCR.

Transfection of CD44s Plasmid

cDNA of CD44s (ORF), ZEB1 (ORF) and MT1-MMP (ORF) were purchased from OriGene. Cells were transfected either with the control vectors or with the CD44s expression vectors by using Lipofectamine 2000 (Invitrogen) according to manufacturer's protocol. Transfection efficiency was examined by qRT-PCR or Western Blot analysis.

Boyden Chamber Matrigel Invasion Assay

Invasion assay was conducted on 24 wells of Matrigel invasion chamber (8 μm, BD Bioscience) following manufacture's instruction. Briefly, 250 μl of 5 $X10^4$ cells in completed medium were added to the upper

well and 0.5 ml of medium was added to lower chamber. The cells were incubated at 37 ^{o}C 5% CO_2 incubator for 22 hours before the invaded cells were analyzed according to manufacturer's protocol.

Collagen Invasion assay

Type I collagen was prepared from rat tail (BD Bioscience) in 0.2% acetic acid to a final concentration of 2.7 mg/ml. To induce gelling, collagen was mixed with 10X MEM and 0.34 N NaOH in an 8:1:1 ratio, and 25 μl of HEPES at 4 ^{o}C, and 1 ml of this mixture was added to the upper well of a 6-well Transwell dish (3-mm pore size; Corning, Inc.). After gelling was complete (45 min at 37 ^{o}C), 1.5 X10^5 cells in complete medium were added to the upper well and 2.5 ml of medium was added to lower chamber. For studying the effect of CD44 or MT1-MMP on the PDAC cell invasion, siRNA transfection or control plasmid transfection was conducted one day before planting cells on the collagen gel. For inhibition experiments, after an additional 24-h incubation period of the planted cells, 5 μg/ml of CD44 antibody (KM114, BD Bioscience) or 50 μM of MT1-MMP inhibitor (NSC405020 , EMD) was added to both the upper and lower wells. Invasion assays were routinely terminated after 3 days. Invasion depths were measured from digitally captured images of hematoxylin and eosin-stained cross-sections.

Surgical orthotopic pancreatic cancer xenograft mouse model

To examine effect of CD44 on pancreatic cancer metastasis, 12 NOD/SCID mice 6-8 weeks old underwent an established surgical method for orthotopic injection of cells into the pancreas. Two groups of cells, control shRNA infected cells and CD44shRNA infected cells, were used for injection. Six mice were used for each group. For this procedure, mice were anesthetized under isoflurane gas. The left upper quadrant abdominal skin and muscle were incised just off the midline and directly above the pancreas to allow visualization of the pancreatic lobes. The pancreas was gently retracted and positioned to allow for direct injection of a 50 μL bolus of 5×10^5 pancreatic cancer cells mixed growth factor reduced matrigel (BD 354230) using a 1 cc syringe with a 30 gauge needle. Successful delivery of cells into the pancreas was observed when a small bubble formed. The pancreas was placed back

within the abdominal cavity and both the muscle and skin layers closed with absorbable surgical sutures. Following recovery from surgery, mice were monitored daily.

Supplemental Table 1. List of Primers.

Sequence Name	Sequence
mAxin2 Forward	GCCAATGGCCAAGTGTCTCT
mAxin2 Reverse	GCGTCATCTCCTTGGGCA
mCD44S Forward	CCA CCA GAG ATC GAG ACT CAT CCA A
mCD44S Reverse	AAG TTG TGG TCA CTC CAC TGT CCT
mCD44T Forward	GGCTCATCATCTTGGCATCT
mCD44T Reverse	TTT CTT CTG CCC ACA CCT TCT CCT
mCdh1 Forward	CAG GTC TCC TCA TGG CTT TGC
mCdh1 Reverse	CTT CCG AAA AGA AGG CTG TCC
mFrmd6 Forward	CAAAGCCATGCAGGACCGT
mFrmd6 Reverse	GATGCCCCAAGTGACTTCGT
mLef1 Forward	AGTGCAGCTATCAACCAGATCCT
mLef1 Reverse	TTTCCGTGCTAGTTCATAGTATTTGG
mMT1-MMP Forward	ACC CTT TGA TGG TGA AGG AGG GTT
mMT1-MMP Reverse	TGG CGG AGG GAT CGT TAG AAT GTT
mSnai1 Forward	AAG ATG CAC ATC CGA AGC
mSnai1 Reverse	ATC TCT TCA CAT CCG AGT GGA
mTwist Forward	CGG GTC ATG GCT AAC GTG
mTwist Reverse	CAG CTT GCC ATC TTG GAG TC
mZeb1 Forward	CAT TTG ATT GAG CAC ATG CG
mZeb1 Reverse	AGC GGT GAT TCA TGT GTT GAG
mGapdh Forward	TGT GTC CGT CGT GGA TCT GA
mGapdh Reverse	CCT GCT TCA CCA CCT TCT TGA
mWwc1 Forward	TGCTGAGGGAAACCAAAGCC
mWwc1 Reverse	CTGGACCATAGGTCGGAGTG
mYap11 Forward	TACTGATGCAGGTACTGCGG
mYap1 Reverse	TCAGGGATCTCAAAGGAGGAC

hCD44S Forward	ATGTGAGTGTCTGGTAGCAGGGAT
hCD44S Reverse	TTGCTCCACCTTCTTGACTCCCAT
hMT1-MMP Forward	CGAGGTGCCCTATGCCTAC
hMT1-MMP Reverse	CTCGGCAGAGTCAAAGTGG