Well Diff

Normal		w	Well Differentiated				Moderate to Poorly Differentiated		
					1 3 3 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			1	
			A		*				
Samples	Ν	Pos (%)	Intensity Score			e	MCS	p Value	
			0	1+	2+	3+		(compared to normal)	
All Cancers	50	37 (74)	13	11	11	15	5.66	0.00962 *	
Normal	8	3 (37.5)	5	3	0	0	0.375		
Types of Cancer									

Mod Diff 18 0 7 5 5.6 0.0009 \* 18 (100) 6 Mod to Poorly Diff 12 3 (25) 9 2.08 0.3178 1 0 2 Others 12 10 (83.3) 4 3 2 3 3.67 0.0317 MCS: Mean Composite Score; Diff: Differentiated; Mod: Moderately; p values determined by a student's t test, \* p values <0.005 were considered significant

0

3

5

0

8.75

0.0002 \*

8 (100)

8

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Supplementary Figure 1. Immunohistochemical expression profile of MUC13 in pancreatic cancer. Tissue microarray: Pancreatic cancer tissue microarray slides were processed for IHC analysis. The array contains 50 pancreatic cancer tissue samples from 25 cancer cases and 8 normal tissue samples. After deparaffinization and blocking, sections were probed with anti-MUC13 MAb (ppz0020) followed by biotinylated secondary antibody incubation. Streptavidin peroxidase and a 3-3'-diamino-benzidine-chromagen system was used for signal detection. MUC13 expression was not detectable in luminal epithelium and stromal tissue in of normal pancreas. Membrane bound and cytoplasmic staining of MUC13 (representative areas indicated by arrows) was observed in pancreatic cancer samples. Table, The mean composite score (MCS) was determined by multiplying the intensity score (0-3) by the percent of cancer cells stained (0-4) for a maximum score of 12 (scored by pathologist, MK). Pancreatic cancer tissue samples had a significantly higher MCS than normal pancreatic tissue. When types of pancreatic cancer were compared, well differentiated and moderately differentiated types had significantly higher MCS compared to normal pancreatic tissue.

**Supplementary Figure 2**. **MUC13 expression in pancreatic cancer cell lines. A,** MUC13 expression at mRNA level as determined by RT-PCR analysis of a panel of pancreatic cancer cell lines. *B*, MUC13 expression at protein level as determined by confocal microscopy analysis. HPAFII and Capan-1 cells show high MUC13 expression while MiaPaca and Panc-1 cells have undetectable levels of MUC13 expression.

Supplementary Figure 3. MUC13 expression alters cell doubling in pancreatic cancer cells. *A*, Cell doubling time in MUC13 overexpressing MiaPaca cells: Cell doubling time was calculated from the cell growth curve during the exponential growth phase by using the formula:  $Td = 0.693t/ln(N_t/N_0)$  as described in the Materials and Method section. MUC13 expression led to reduced cell doubling times. *B*, Cell doubling time in MUC13 knockdown HPAFII cells: Suppression of MUC13 expression caused an increase in cell doubling times. Mean  $\pm$  SE; n=3.

Supplementary Figure 4. MUC13 expression enhances tumorigenic characteristics in Panc-1 pancreatic cancer cells. A, RT-PCR and confocal microscopy analysis: Panc-1 cells were transfected with full-length MUC13. For RT-PCR, RNA samples isolated from pancreatic cancer cell lines were reverse transcribed and then cDNA samples were PCR amplified. HPAFII and MUC13 transfected Panc-1 cells (Panc-1-M13B and Panc-1-M13D) showed MUC13 overexpression in RT-PCR analysis (left panel). For RT-PCR analysis, GAPDH was used as an internal control. The MUC13 expression in Panc-1 derived cells was confirmed by confocal microscopy (right panel). Note: MUC13 expression (green) is evident only in MUC13transfected cells (Panc-1-M13D has greater MUC13 expression than Panc-1-M13B) but not in vector alone-transfected (Panc-1-V4) cells. Original magnification, 400x. **B**, Cell proliferation assav: Cells (2 x10<sup>4</sup>) were seeded in six-well plates. Cell number was determined after 24, 48, 72 and 96 h by automated cell counter. Mean  $\pm$  SE; n=3; \* p<0.05. MUC13 expressing clones (Panc-1-M13D) showed significantly higher cellular proliferation compared to vector control (Panc-1-V4) cells. C. Anchorage-independent soft agar colony forming assays: Cells (2  $x10^4$ ) were seeded in a 6-well plate in 1 ml of 0.3% complete media-agar, cultures were maintained in CO<sub>2</sub> incubator for 15 days, six photographs were taken from each well and colonies were counted. Mean  $\pm$  SE; n=3; \* p<0.05. MUC13 expressing cells (Panc-1-M13D) formed significantly higher number of colonies compared to the MUC13 null vector control (Panc-1-V4) cells.

**Supplementary Figure 5**. **MUC13 expression alters HER2 and p53 expression in xenograft tumors**. *A*, IHC analysis of xenograft tumors generated from MUC13 overexpressing (MP4) and MUC13 null (MPPC) MiaPaca pancreatic cancer cells in nude mice. Sections were stained for MUC13, HER2 and p53 as indicated. *Note:* HER2 expression is increased in MUC13- overexpressing tumors (MP4) compared to vector control (MPPC) tumors. Conversely, the level of p53 is decreased in MUC13-overexpressing tumors. *B*, IHC analysis of xenograft tumors generated from MUC13 knockdown (shMUC13D) and MUC13 expressing (sh-V-GFP) HPAFII pancreatic cancer cells in nude mice. An increased p53 and a decreased HER2 expression is evident in MUC13 knockdown tumors (shMUC13D) compared to vector control (sh-V-GFP) tumors. Original magnifications 200X.