Supplementary Fig. S1. Schematic representation of the study design used in this project. (Top Left) The characteristic spike of hypermethylated genes defined by treatment of cells with DAC or TSA consists of two tiers, with distinct features. The top tier (TT) of genes was identified as a zone in which gene expression did not increase with TSA (1.4 fold) and displayed no detectable expression in wild-type cells, but increased greater than 2-fold with DAC treatment. The next tier (NT) of genes was identified as a cluster of genes for which expression changes of TSA and wild type were identical to those in the top tier, but increased between 1.4-fold and 2-fold with DAC treatment. Gene expression validation by qPCR and MSP were performed in four different pancreatic cancer cell lines (Panc1, MIA-PaCa2, PL45, and Capan1). (Top right) Shared candidate hypermethylated genes in pancreatic cancer cell lines. We identified a total of 1,427 unique genes in all four cell lines with expression changes falling within the criteria of top-tier category. Overlaps in gene expression changes among two, three, or four cell lines are indicated. These range from 285 genes shared among two cell lines to 16 genes that were shared among all four cell lines. Eight of these genes were chosen to be validated by MSP in pancreatic tissues. The top 2 genes, *ADAMTS1* and *BNC1* were chosen to explore the correlation between methylation and expression in fresh pancreas tissues and the Cancer Genome Atlas. Finally, methylation of *ADAMTS1* and *BNC1* were explored in the serum of 42 patients with pancreatic cancer and 26 patients with a normal pancreas.

Supplementary Figure S2. mRNA and Protein Expression in *BNC1* Overexpression System A) qPCR measuring *BNC1* overexpression (*BNC1* OE) in Panc1 (red) and MIA-PaCa2 (blue) as compared to that of normal pancreas (green). **B)** qPCR measuring mRNA levels of *BNC1* overexpression (*BNC1* OE) as compared to that of mock-treated cells (Mock) and that of the empty vector (Empty vector) in Panc1 (red) and MIA-PaCa2 (blue). **C)** Western blot analysis of BNC1 protein expression in Panc-1 and MIA-PaCa2 cells. Samples were analyzed by Western blotting using anti-*BNC1* antibodies. Mock indicates no treatment. Empty vector and

subcloned full-length BNC1 cDNA vector were transfected in Panc-1 and MIA-PaCa2 cells and incubated for 48 hours.

Supplemental Figure S3. Kaplan-Meier Curve of Overall Survival Characterized by *ADAMTS1* Methylation Overall survival was measured for patients with methylated and unmethylated *ADAMTS1*. This was significant by log-rank analysis (p=0.03) as well as by univariate Cox regression analysis (*ADAMTS1* Unmethylated as reference, *ADAMTS1* Methylated- Odds Ratio 1.6; Confidence Interval 1.03-2.52; p=0.03).

Supplemental Materials and Methods

***Western blot analysis*:** For Western blot analysis, cells were harvested, washed, and lysed in extraction buffer (50 mM Tris, 10% glycerol, 150 mM NaCl, pH 8.0). Proteins were separated by electrophoresis using precast a 4–20% gradient Tris glycine gel (Invitrogen), and transferred to a nitrocellulose membrane filter (Millipore). The membranes were blocked with 5% nonfat milk. Primary antibodies used for Western blot analysis were *BNC1* (Thermo Scientific), and β-actin (Sigma). Protein bands were visualized by using the enhanced chemiluminescence system, Fusion FX5 (Vilber Lourmat).

***Kaplan-Meier Analysis*:** To determine if *ADAMTS1* was able to predict overall survival, a Kaplan-Meier analysis of all patients with PanIN (n=20) and pancreatic cancers (n=123) was performed. Survival was calculated as date from surgery to date of death. Patients lost to follow up were censored at their last visit. A log-rank analysis was performed to determine the difference in survival between patients with methylated *ADAMTS1* and unmethylated *ADAMTS1*. Univariate Cox regression models estimated relative hazards between *ADAMTS1* methylation status and all-cause mortality. Results of Cox regression were reported as hazard ratios (HR) with 95% confidence intervals (CIs). Statistical analyses and graphic renderings were performed R: A Language and Environment for Statistical Computing.

CA 19-9 Analysis: Pre-operative CA 19-9 levels were investigated in our patient population. 45.1% of patients in our cohort had pre-operative CA 19-9 levels measured. The range for normal CA 19-9 at our institution is 0-36 U/mL and values greater than 36 was considered elevated and abnormal.

The median CA 19-9 for all patients was 114.5 U/mL. The rate of abnormal and elevated CA 19-9 in all invasive cancers was 70%. This increased with increasing stage. 52% of Stage I cancers had an elevated CA 19-9. This increased to 73.7% of Stage II cancers and 100% of

Stage III and IV cancers. In all instances the combination of methylation of *ADAMTS1* and *BNC1* showed improved sensitivity compared to CA 19-9.