

## Supplementary Materials for

#### Detection of early pancreatic ductal adenocarcinoma with thrombospondin-2 and CA19-9 blood markers

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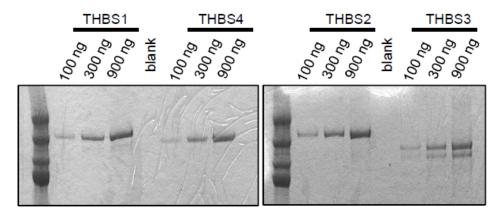
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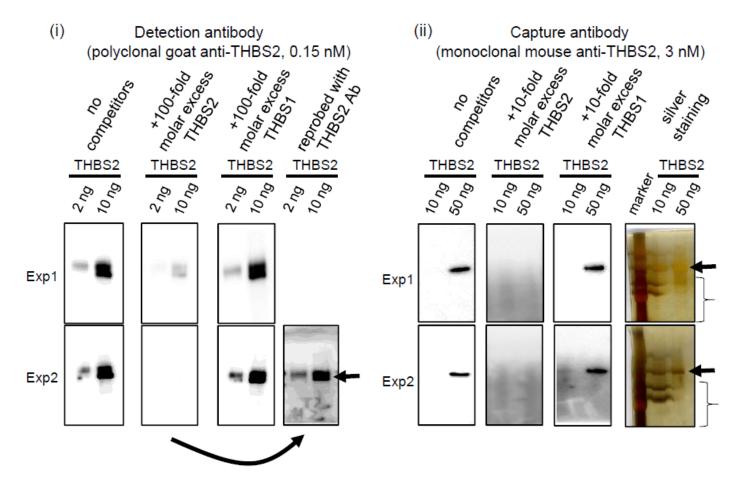
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A. SDS-PAGE confirmation of expected relative sizes of recombinant THBS1, THBS2, THBS3, and THBS4 proteins obtained from Bio-Techne, Inc..



B. ELISA detection and capture antibodies for THBS2 are competed by THBS2, not by THBS1



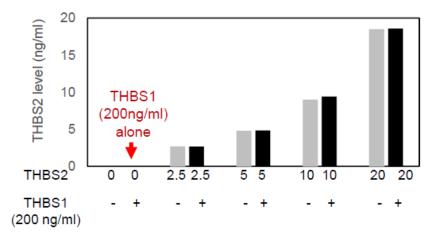
## Fig. S1. Validation of cross-reactivity of antibodies enclosed in THBS2 ELISA with THBS1 by Western blot.

A. SDS-PAGE confirmation of expected relative sizes of recombinant THBS1, THBS2, THBS3, and THBS4 proteins obtained from Bio-Techne, Inc. The company had validated the specificity of the THBS2 ELISA kit with these recombinant proteins. Because of prior claims that THBS1 is down-regulated in PDAC (see text), we assessed the cross-reactivity of THBS1 against the detection and capture antibodies in the THBS2 ELISA kit in duplicate experiments (Exp1 and Exp2).

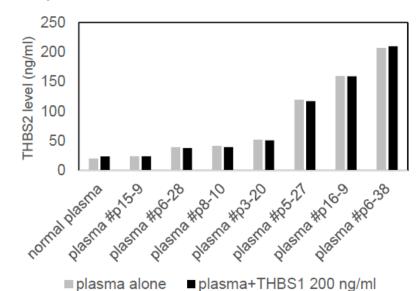
B. ELISA detection and capture antibodies for THBS2 are competed by THBS2, not by THBS1. (i) Samples (0.15 nM) of goat polyclonal anti-THBS2 detection antibody were used with no competitor or with a 100-fold molar excess of recombinant proteins THBS2 or THBS1 for 30 min at room temperature. The reactions were applied to Western blot membranes with 2 or 10 ng THBS2 in separate lanes. The excess THBS2 competes for the signal while the excess THBS1 does not. To demonstrate that the membrane for the THBS2 competition did have THBS2 protein, the first antibody reaction was stripped and the blot was re-probed (arrow) with the THBS2 antibody without competitor.

(ii) Samples (3 nM) of mouse monoclonal anti-THBS2 capture antibody were used with no competitor or with a 10-fold molar excess of recombinant proteins THBS2 or THBS1 for 30 min at room temperature. All THBS2 proteins were run in a gel and transferred into the same membrane. The membrane was blocked and divided into three pieces before applying the mixture of detection antibody and competitors. The reactions were applied to Western blot membranes with 10 or 50 ng THBS2 in separate lanes. The excess THBS2 competes for the signal while the excess THBS1 does not. To demonstrate that the membrane for the THBS2 competition did have THBS2 protein, the blot was stripped and stained with silver. Arrows point to recombinant THBS2 evident on the blot (signals were saturated at 10 ng). Brackets indicate the marker bands that spilled over from neighboring lanes. Note that the monoclonal capture antibody has less sensitivity than the polyclonal detection antibody.

A. Presence of excess THBS1 has no effect on ELISA detection of recombinant THBS2 proteins



B. Presence of excess THBS1 has no effect on ELISA detection of THBS2 in human plasma

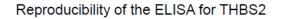


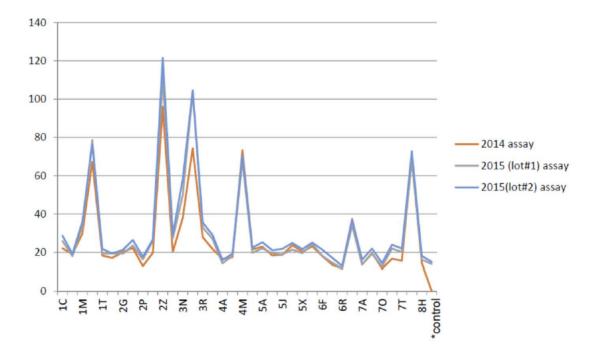
THBS2 (ng/ml)					plasma #p3-20	plasma #p5-27	plasma #p16-9	plasma #p6-38
plasma alone	20.4	24.2	39.3	41.7	51.9	119.6	159.6	207.1
plasma+THBS1 200ng/ml	23.7	24.3	38.3	39.8	51.2	117.4	159.3	210.0
CV(%)	10.7	0.3	1.9	3.3	0.9	1.3	0.1	1.0

#### Fig. S2. Validation of cross-reactivity and interference of THBS1 in THBS2 ELISA.

(A) Presence of excess THBS1 has no effect on ELISA detection of recombinant THBS2 proteins. To determine whether presence of THBS1 interferes with the THBS2 ELISA kit, 200 ng/ml of recombinant THBS1 protein was spiked into various concentrations of recombinant THBS2 proteins (0 ng/ml to 20 ng/ml) and subjected to THBS2 ELISA assays. Grey bars indicate THBS2 protein alone and black bars indicate THBS2 proteins spiked with THBS1. The differences in THBS2 detection are negligible (less than 5% CV).

(B) Presence of excess THBS1 has no effect on ELISA detection of THBS2 in human plasma. ELISA assays were performed on Phase 2b plasma samples randomly picked to exhibit a range of THBS2 concentrations, along with human normal pooled plasma, with and without 200 ng/ml THBS1 protein. Grey bars show the plasma alone and black bars show the plasma with a 200 ng/ml of THBS1. The differences in THBS2 concentration in Phase 2b plasmas between absence and presence of THBS1 protein (200 ng/ml) were less than 5% CV, while the very low concentrations in the commercial normal pooled plasma exhibited a 10% CV). Primary data are shown at bottom.

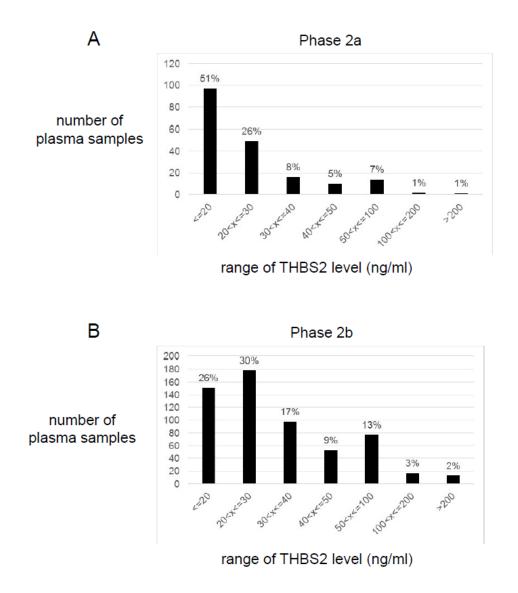




#### Fig. S3. Reproducibility of the ELISA for THBS2.

The plot shows primary ELISA data in nanograms THBS2 per ml of plasma for a set of Phase 2a samples. The signals were determined from a standard curve as described in the Materials and Methods. The same samples were assessed once in 2014 and twice in 2015, using different lots for each assay. The two assays in 2015 included a commercial plasma sample mixed from normal, healthy individuals (\*denoted "control").

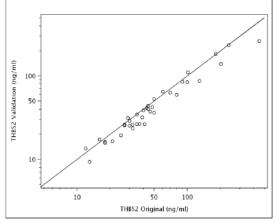
#### Distribution of THBS2 values in Phase 2 samples



#### Fig. S4. Distribution of THBS2 values in phase 2 samples.

Data for Phase 2a (A) and Phase 2b (B) plasma samples in ELISA were rank-ordered and divided into 7 groups based upon THBS2 protein concentrations. The frequency of samples in each group is shown.

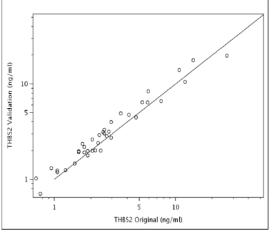
Cross-validation tests, performed one year apart, of THBS2 levels in the same set of plasmas as determined in different laboratories



A. Scatterplot of original THBS vs. validation THBS2 for N=38 samples

Correlation Coefficient, Pearson=0.950 Spearman=0.968

B. Scatterplot of original THBS vs. validation THBS2 for N=38 samples (re-scaled data)



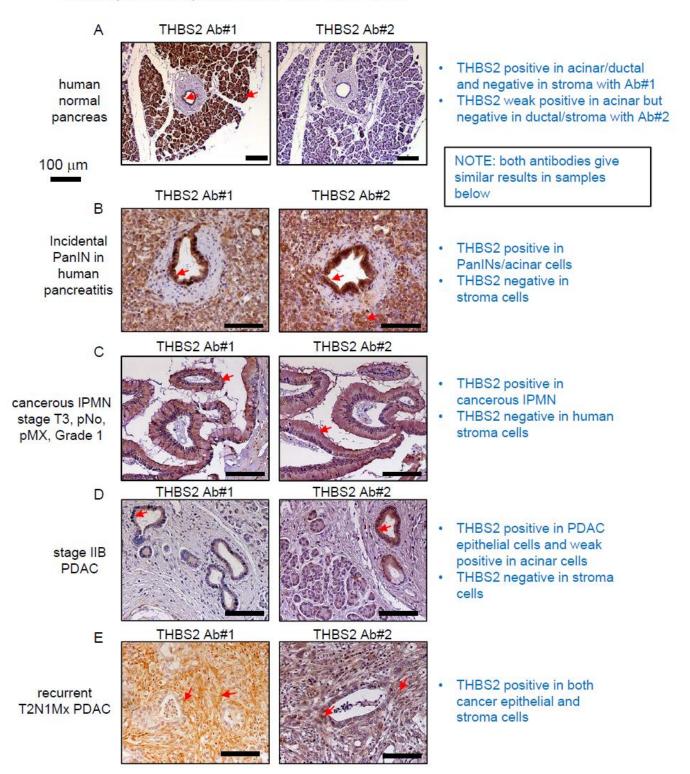
Correlation Coefficient, Pearson=0.950 Spearman=0.968

# Fig. S5. Cross-validation tests, performed 1 year apart, of THBS2 concentrations in the same set of plasmas as determined in different laboratories.

A subset of Phase 2b samples were cross-validated in a blinded fashion, in an independent lab that was provided only the relevant methods section of this paper and the manufacturer's instructions for methodology. (A) Scatterplot of original THBS2 values versus cross-validated THBS2 values, as determined one year later with a different lot number of reagents, in subset of Phase 2b plasma samples (n=38). The X-axis indicates the THBS2 values originally obtained in 2015 and Y-axis indicates the THBS2 values cross-validated by an independent lab in 2016. The Pearson correlation coefficient is 0.95 and the Spearman coefficient is 0.968, indicating a strong correlation between the original and validated assays.

(B) Scatterplot of original THBS2 versus cross-validated THBS2 values rescaled by commercial human normal pooled plasma. The average THBS2 value in the normal pooled plasma control in the original assay, where our set point was derived, was 17 ng/ml and the average THBS2 value in the normal pooled plasma control in the cross-validated samples was 13.25. Thus we re-plotted the data, as shown, by dividing the cross-validation samples by the scaling factor as described in the text. The re-scaled THBS2 values in cross-validation had a negilable effect on the correlation coefficiency, indicating a robust assay.

Representative immunohistochemistry (IHC) images of THBS2 in human normal pancreas, pancreatitis, and PDAC tissue



# Fig. S6. Representative immunohistochemistry images of THBS2 in human normal pancreas, pancreatitis, and PDAC tissue.

Two "IHC quality" THBS2 antibodies were tested: Ab#1 (rabbit polyclonal THBS2 antibody, dilution 1:100, TA590658, Origene) and Ab#2 (Goat polyclonal THBS2 antibody, dilution 1:25, sc-7655, Santa Cruz). Red arrows designate positive cells. Scale bar equals 100 microns in each image.

(A) IHC in human normal pancreas. THBS2 was detected in acinar and ductal compartment but not in stroma with Ab#1. However, THBS2 was barely detected in the acinar compartment and not in ducts or stroma with Ab#2.

(B) IHC in human pancreatitis. THBS2 was detected in incidental PanINs in pancreatitis and acinar cells but not in stroma cells with both antibodies.

(C) IHC in cancerous IPMN stage T3, pNo, pMX, Grade 1. THBS2 was detected in cancerous IPMN but not in stromal cells with both antibodies.

(D) IHC in PDAC stage 2b. THBS2 was detected in PDAC epithelial cells and weakly labeled in acinar cells, but not in stroma cells with both antibodies.

(E) IHC in recurrent T2N1Mx PDAC. THBS2 was detected in both cancer epithelial and stroma cells with both antibodies. Brown colors indicate the compartments labeled with THBS2 in the left panel.

## Table S1. List of 53 proteins secreted or released from 10-22 cell, PanIN-stage lesions that are at lowabundance in healthy human plasma proteome and RNA-seq databases.

IPI number	Protein	PDAC Network from	Identified in plasma protein DB	Identified in plasma MOPED database	ELISA kits available
		Kim et al. 2013 Cell Reports	(as of 2014)	As of 2014	
IPI00152881	SHROOM3	TGFb/Integrin	yes	0.1 nmol	no
IPI00215893	HMOX1	TGFb/Integrin	yes	0 nmol	no
IPI00007960	*PERIOSTIN	TGFb/Integrin	yes	no information	yes
IPI00013405	MMP10	TGF/Integrin	no	0 nmol	yes
IPI00027780	MMP-2	TGF/Integrin	no	2 nmol	yes
IPI00018769	THBS2	TGF/Integrin	yes	no information	yes
IPI00009841	EWSR1	TGFb/Integin	yes	no information	no
IPI00005776	NOD1	TGF/Integrin	yes	1 nmol	no
IPI00220986	ADAMTS9	TGF/Integrin	yes	1 nmol	no
IPI00022443	*AFP	TGF/Integrin	yes	1 nmol	yes
IPI00247295	SYNE1	TGF/Integrin	yes	0.2 nmol	no
IPI00239405	SYNE2	TGFb/Integrin	yes	no information	no
IPI00008315	EPHB1	TGFb/Integrin	yes	0 nmol	no
IPI00218292	UFD1L	TGFb/Integrin	no	0 nmol	no
IPI00002901	TEAD1	TGFb/Integrin	no	0 nmol	no
IPI00027280	TOP2B	TGFb/Integrin	yes	0.1-1 nmol	no
IPI00217185	RYR3	TGF/Integrin	yes	0.8 nmol	no
IPI00166612	CMYA5	TGF/Integrin	yes	0 nmol	no
IPI00221255	MYLK	TGF/Integrin	yes	0.2 nmol	no
IPI00251161	KIAA1109	RAS/p53/JUN/ CTNB1	yes	0.1 nmol	no
IPI00398020	ODZ3	RAS/p53/JUN/ CTNB1	yes	no information	no
IPI00235481	PMFBP1	RAS/p53/JUN/ CTNB1	yes	0.1 nmol	no
IPI00289329	EPHB3	RAS/p53/JUN/ CTNB1	yes	no information	no
IPI00177498	LIMCH1	RAS/p53/JUN/ CTNB1	yes	no information	no
IPI00159322	TCF20	RAS/p53/JUN/ CTNB1	yes	0.1 nmol	no
IPI00847609	SVEP1	no network	yes	0.1 nmol	no
IPI00396634	KIAA1671	no network	no	0 nmol	no
IPI00292836	KIAA1529	no network	yes	0 nmol	no
IPI00896378	GNN	no network	no	no information	no
IPI00420019	DOS	no network	no	0.5 nmol	no
IPI00293887	STARD8 (DLC3)	no network	yes	0 nmol	no
IPI00183041	SCN8A	no network	yes	0.5 nmol	no
IPI00143753	U2SURP	no network	no	0 nmol	no
IPI00031104	TCHP	no network	yes	1 nmol	no
IPI00012829	RAD51C	no network	yes	no information	no
IPI00024804	ATP2A1	no network	yes	0 nmol	no
IPI00377214	NLRX1	no network	no	0 nmol	no
IPI00028833	ZNF160	no network	yes	1 nmol	no
IPI00645947	RTTN	no network	no	0 nmol	no
IPI00328762	ABCA13	no network	yes	0 nmol	no

IPI00288940	OBSCN	HNF4a	yes	0.1 nmol	no
IPI00011385	LOXL3	HNF4a	yes	0 nmol	no
IPI00029046	MLEC	HNF4a	yes	0 nmol	no
IPI00002127	DNAH1	HNF4a	yes	0 nmol	no
IPI00152653	DNAH5	HNF4a	yes	1 nmol	no
IPI00412106	DNAH12	HNF4a	yes	1 nmol	no
IPI00888430	DNAH17	HNF4a	no	0 nmol	no
IPI00396218	SCYL2	HNF4a	yes	0 nmol	no
IPI00303300	FKBP10	HNF4a	no	0 nmol	no
IPI00002320	FLRT3	HNF4a	yes	0 nmol	no
IPI00007256	ZHX2(AFR1)	HNF4a	yes	0 nmol	no
IPI00375560	ZNF804A	HNF4a	no	0.1 nmol	no
IPI00019884	ACTN2	HNF4a	yes	0.1 nmol	no

\* AFP and PERIOSTIN were not chosen because of their indication in hepatocellular carcinoma and breast cancer, respectively.

Table S2A	sequence	presence of peptides by search engine pFind 2.8		area under t THBS2 spec original ms1,		THBS2 ratio, cancer/normal	*adjusted ratio of THBS2 by total PSM	FDR
		Pooled normal controls (n=10)	Pooled PDAC patients (n=10)	Pooled normal controls (n=10)	Pooled PDAC patients (n=10)	-	PSM	
peptide 1 (THBS2 specific)	TRNMSACWQDGR	presence	n.d (not- determined)	55814	261090	4.68	3.04	FDR 5%
peptide 2 (THBS2 specific)	VCNSPEPQYGGK	n.d	presence	0	792162	n.d.	n.d.	FDR 5%
peptide 3 (THBS1/THBS2 shared)	FYVVMWK	presence	n.d	4599455	13383548	2.9	1.88	FDR 5%
peptide4 (THBS1/THBS2 shared)	NALWHTGNTPGQVR	n.d	presence	8373610	21837763	2.6	1.69	FDR 5%
Total number of PSM (peptide spectrum matches)				22330	34402	1.54		FDR 5%

Table S2A. Peptides searched with pFind 2.8 at 5% FDR

\* Adjusted ratio of THBS2 was calculated from the original ratio THBS2 dividing by a normalization factor (1.54) acquired by dividing the total number of peptide spectrum matches (PSM) in cancer by the total PSM number in the normal sample. The ratio for peptide 2 and the overall average ratio of THBS2 was not computed because peptide 2 was only observed in the PDAC pooled sample.

Table S2B. Peptides searched with pFind3.0 at 5% FDR

Table S2B	sequence	presence of per engine	otides by search pFind 3.	FDR
		Pooled normal controls (n=10)	Pooled PDAC patients (n=10)	
peptide 1 (THBS2 specific)	TRNMSACWQDGR	n.d	n.d	FDR 5%
peptide 2 (THBS2 specific)	VCNSPEPQYGGK	n.d	presence	FDR 5%
peptide 3 (THBS1/THBS2 shared)	FYVVMWK	presence	n.d	FDR 5%
peptide4 (THBS1/THBS2 shared)	NALWHTGNTPGQVR	presence	presence	FDR 5%

Table S2C	sequence		by search engine pFind .0	FDR	
		Pooled normal controls (n=10)	Pooled PDAC patients (n=10)		
peptide 1 (THBS2 specific)	TRNMSACWQDGR	n.d	n.d	FDR 1%	
peptide 2 (THBS2 specific)	VCNSPEPQYGGK	n.d	presence	FDR 1%	
peptide 3 (THBS1/THBS2 shared)	FYVVMWK	n.d	n.d	FDR 1%	
peptide4 (THBS1/THBS2 shared)	NALWHTGNTPGQVR	presence	presence	FDR 1%	

### Table S2C. Peptides searched with pFind3.0 at 1% FDR

### Table S3. Range and median values of THBS2 and CA19-9 in this study.

THBS2	n	median	min	P_25	P_75	max
Adenocarcinoma	288	37.171	11.590	25.6620	60.80	451.40
Stage I/II	152	35.291	11.590	25.582	55.41	451.40
Stage III/IV	136	40.797	11.699	26.621	67.10	269.71
Controls	230	19.298	6.425	16.521	24.10	73.39
IPMN, no Adeno	115	24.666	10.142	19.426	32.55	72.52
Islet Cell	30	30.033	13.872	22.212	45.67	433.46
Pancreatitis	83	24.094	6.458	17.949	31.74	209.12

Ca19-9	n	median	min	P_25	P_75	max
Adenocarcinoma	288	219.500	0.599	55.9000	1137.00	10000.00
Stage I/II	152	141.000	0.599	31.800	442.00	10000.00
Stage III/IV	136	615.500	0.600	103.000	3973.00	10000.00
Controls	230	8.650	0.599	5.400	14.00	136.00
IPMN, no Adeno	115	12.200	0.600	6.700	21.30	56.20
Islet Cell	30	11.200	0.599	5.100	29.10	461.00
Pancreatitis	83	14.700	0.600	8.500	32.20	833.00

## Table S4. Impact of excluding stage IIB (and unspecified stage II) subjects.

PDAC vs.		CA	19-9(≥ 55	)	THBS2				CA19-9(≥ 55) + THBS2			
Healthy Controls	N	AUC	95%	6 CI	AUC	95% CI			AUC	95%	6 CI	p-value
Validation Phase 2a												
Stage I/IIA/II/IIB	58/80	0.845	0.80	0.89	0.832	0.78	0.89		0.946	0.92	0.98	0.0067
Stage I/IIA/II	37/80	0.851	0.80	0.91	0.810	0.74	0.89		0.937	0.90	0.98	0.0767
Stage I/IIA	21/80	0.905	0.85	0.97	0.819	0.73	0.91		0.919	0.85	1.00	0.7058
Validation Phase 2b												
Stage I/IIA/II/IIB	88/140	0.834	0.79	0.87	0.887	0.85	0.92		0.960	0.94	0.98	<0.0001
Stage I/IIA/II	62/140	0.824	0.78	0.87	0.861	0.82	0.90		0.950	0.93	0.97	0.0002
Stage I/IIA	25/140	0.773	0.70	0.86	0.905	0.86	0.95		0.958	0.93	0.98	0.0005

### Table S5A. THBS2 values by sex and diabetes mellitus status.

IPMN         Female         57         25.8860         12.096         20.7010         32.2340         70.887           IPMN         Male         58         24.1590         10.142         18.5610         33.2390         72.515           PNET         Female         8         29.3040         21.391         24.1965         45.8290         100.724           PNET         Male         22         32.7955         13.872         19.1610         45.6740         433.460           Pancreatitis         Female         36         21.0220         6.458         17.6355         30.4205         67.388           Pancreatitis         Male         46         25.2530         11.409         19.0730         36.3010         209.117           Adenocarcinoma Stage I/II         Male         92         31.8888         11.590         22.5440         51.6485         451.397           Adenocarcinoma Stage III/IV         Female         60         40.5035         15.698         26.2460         67.0990         218.442           Adenocarcinoma Stage III/IV         Male         76         40.8955         11.699         27.1895         67.2140         26.9711           Controls         Female         101         20.								
IPMN PNET PNETMale5824.159010.14218.561033.239072.516PNET PNETMale2232.795513.87219.161045.6740433.460Pancreatitis PancreatitisFemale3621.02206.45817.635530.420567.386Pancreatitis Adenocarcinoma Stage I/II Adenocarcinoma Stage III/IVFemale6038.007015.18230.207061.8390216.687Adenocarcinoma Stage III/IV ControlsFemale6040.503515.69826.246067.0990218.448Adenocarcinoma Stage III/IV ControlsFemale10120.790012.01217.233026.268065.573ControlsFemale10120.790012.01217.233026.268065.57373.924IPMN PNETNo DM9523.880010.14219.367029.962060.2873IPMN PNETNo DM2034.169513.76424.613551.563072.515PNET PNETNo DM2028.672013.87220.276062.8445433.460PNET PancreatitisNo DM7224.55106.45818.112330.4205209.117Pancreatitis PNETDM1026.171511.63718.447042.588077.424Adenocarcinoma Stage I//I PancreatitisNo DM7224.55106.45818.112330.4205209.117Pancreatitis PancreatitisDM1026.171511.637	group		n	median	min	P_25	P_75	max
PNET         Female         8         29.3040         21.391         24.1965         45.8290         100.724           PNET         Male         22         32.7955         13.872         19.1610         45.6740         433.460           Pancreatitis         Female         36         21.0220         6.458         17.6355         30.4205         67.386           Pancreatitis         Male         46         25.2530         11.409         19.0730         36.3010         209.117           Adenocarcinoma Stage I/II         Male         92         31.8888         11.590         22.5440         51.6485         451.397           Adenocarcinoma Stage III/IV         Female         60         40.5035         15.698         26.2460         67.0990         218.448           Adenocarcinoma Stage III/IV         Male         76         40.8955         11.699         27.1895         67.2140         269.711           Controls         Female         101         20.7900         12.012         17.2330         26.2680         65.573           Controls         Male         129         18.1210         6.425         15.4790         22.3270         73.392           IPMN         DM         92         23.	IPMN	Female	57	25.8860	12.096	20.7010	32.2340	70.881
PNET       Male       22       32.7955       13.872       19.1610       45.6740       433.460         Pancreatitis       Female       36       21.0220       6.458       17.6355       30.4205       67.388         Pancreatitis       Male       46       25.2530       11.409       19.0730       36.3010       209.117         Adenocarcinoma Stage I/II       Female       60       38.0070       15.182       30.2070       61.8390       21.688         Adenocarcinoma Stage I/II       Male       92       31.8888       11.590       22.5440       51.6485       451.397         Adenocarcinoma Stage III/IV       Male       76       40.8955       11.699       27.1895       67.2140       269.714         Adenocarcinoma Stage III/IV       Male       76       40.8955       11.699       27.1895       67.2140       269.714         Controls       Female       101       20.7900       12.012       17.2330       26.2680       65.573         Controls       Male       129       18.1210       6.425       15.470       22.3270       73.392         IPMN       No DM       95       23.8800       10.142       19.3670       29.9620       60.287	IPMN	Male	58	24.1590	10.142	18.5610	33.2390	72.515
Pancreatitis Pancreatitis       Female       36       21.0220       6.458       17.6355       30.4205       67.388         Adenocarcinoma Stage I/II Adenocarcinoma Stage II/IV       Female       60       38.0070       15.182       30.2070       61.8390       21.6687         Adenocarcinoma Stage II/IV Adenocarcinoma Stage III/IV       Female       60       38.0070       15.182       30.2070       61.8390       21.6687         Adenocarcinoma Stage II/IV Adenocarcinoma Stage III/IV       Male       92       31.8888       11.590       22.5440       51.6485       451.397         Controls Controls       Female       60       40.5035       15.698       26.2460       67.0990       218.448         Male       76       40.8955       11.699       27.1895       67.2140       269.711         Controls       Male       76       40.8955       11.699       27.1895       67.2140       269.711         Male       129       18.1210       6.425       15.4790       22.3270       73.392         IPMN       No DM       95       23.8800       10.142       19.3670       29.9620       60.287         PNET       No DM       20       28.6720       13.872       20.2760 <t< td=""><td>PNET</td><td>Female</td><td>8</td><td>29.3040</td><td>21.391</td><td>24.1965</td><td>45.8290</td><td>100.724</td></t<>	PNET	Female	8	29.3040	21.391	24.1965	45.8290	100.724
Pancreatitis       Male       46       25.2530       11.409       19.0730       36.3010       209.117         Adenocarcinoma Stage I/II       Female       60       38.0070       15.182       30.2070       61.8390       216.687         Adenocarcinoma Stage I/II       Male       92       31.8888       11.590       22.5440       51.6485       451.397         Adenocarcinoma Stage III/IV       Female       60       40.5035       15.698       26.2460       67.0990       218.448         Adenocarcinoma Stage III/IV       Controls       Female       60       40.8955       11.699       27.1895       67.2140       269.711         Controls       Controls       Male       76       40.8955       11.699       27.1895       67.2140       269.711         Controls       Male       76       40.8955       11.699       27.1895       67.2140       269.711         Controls       Male       129       18.1210       6.425       15.4790       22.3270       73.392         IPMN       No DM       95       23.8800       10.142       19.3670       29.9620       60.287         PNET       No DM       20       28.6720       13.872       20.2760       62.8445<	PNET	Male	22	32.7955	13.872	19.1610	45.6740	433.460
Adenocarcinoma Stage I/II       Female       60       38.0070       15.182       30.2070       61.8390       216.687         Adenocarcinoma Stage II/IV       Male       92       31.8888       11.590       22.5440       51.6485       451.397         Adenocarcinoma Stage III/IV       Female       60       40.5035       15.698       26.2460       67.0990       218.449         Adenocarcinoma Stage III/IV       Controls       Female       60       40.8955       11.699       27.1895       67.2140       269.711         Controls       Controls       Male       76       40.8955       11.699       27.1895       67.2140       269.711         Female       101       20.7900       12.012       17.2330       26.2680       65.573         Male       129       18.1210       6.425       15.4790       22.3270       73.392         IPMN       No DM       95       23.8800       10.142       19.3670       29.9620       60.287         PNET       No DM       20       28.6720       13.872       20.2760       62.8445       433.460         PNET       DM       10       39.3765       13.967       23.1640       44.9590       111.390 <t< td=""><td>Pancreatitis</td><td>Female</td><td>36</td><td>21.0220</td><td>6.458</td><td>17.6355</td><td>30.4205</td><td>67.388</td></t<>	Pancreatitis	Female	36	21.0220	6.458	17.6355	30.4205	67.388
Adenocarcinoma Stage I/II       Male       92       31.8888       11.590       22.5440       51.6485       451.397         Adenocarcinoma Stage III/IV       Female       60       40.5035       15.698       26.2460       67.0990       218.448         Adenocarcinoma Stage III/IV       Male       76       40.8955       11.699       27.1895       67.2140       269.714         Controls       Female       101       20.7900       12.012       17.2330       26.2680       65.573         Controls       Male       129       18.1210       6.425       15.4790       22.3270       73.392         IPMN       No DM       95       23.8800       10.142       19.3670       29.9620       60.287         IPMN       DM       20       34.1695       13.764       24.6135       51.5630       72.515         PNET       No DM       20       28.6720       13.872       20.2760       62.8445       433.460         PNET       DM       10       39.3765       13.967       23.1640       44.9590       111.390         Pancreatitis       DM       10       26.1715       11.637       18.4470       42.5880       77.424         Adenocarcinoma Stage I//I<	Pancreatitis	Male	46	25.2530	11.409	19.0730	36.3010	209.117
Adenocarcinoma Stage III/IV       Female 60       40.5035       15.698       26.2460       67.0990       218.443         Adenocarcinoma Stage III/IV       Male 76       40.8955       11.699       27.1895       67.2140       269.714         Controls       Female 101       20.7900       12.012       17.2330       26.2680       65.573         Controls       Male 129       18.1210       6.425       15.4790       22.3270       73.392         IPMN       No DM       95       23.8800       10.142       19.3670       29.9620       60.287         IPMN       DM       20       34.1695       13.764       24.6135       51.5630       72.515         PNET       No DM       20       28.6720       13.872       20.2760       62.8445       433.460         PNET       DM       10       39.3765       13.967       23.1640       44.9590       111.390         Pancreatitis       DM       10       26.1715       11.637       18.4470       42.5880       77.424         Adenocarcinoma Stage I//I       No DM       102       35.0490       11.590       25.5830       57.6440       248.952         Adenocarcinoma Stage II//IV       No DM       108       3	Adenocarcinoma Stage I/II	Female	60	38.0070	15.182	30.2070	61.8390	216.687
Adenocarcinoma Stage III/IV       Male       76       40.8955       11.699       27.1895       67.2140       269.711         Controls       Female 101       20.7900       12.012       17.2330       26.2680       65.573         Male       129       18.1210       6.425       15.4790       22.3270       73.392         IPMN       No DM       95       23.8800       10.142       19.3670       29.9620       60.287         IPMN       DM       20       34.1695       13.764       24.6135       51.5630       72.515         PNET       No DM       20       28.6720       13.872       20.2760       62.8445       433.460         PNET       DM       10       39.3765       13.967       23.1640       44.9590       111.390         PNET       DM       10       39.3765       13.967       23.1640       44.9590       111.390         Pancreatitis       No DM       72       24.5510       6.458       18.1123       30.4205       209.117         DM       10       26.1715       11.637       18.4470       42.5880       77.424         Adenocarcinoma Stage I/II       No DM       102       35.0490       11.590       25.5830	Adenocarcinoma Stage I/II	Male	92	31.8888	11.590	22.5440	51.6485	451.397
Controls ControlsFemale 10120.790012.01217.233026.268065.573Male 12918.12106.42515.479022.327073.392IPMN IPMNNo DM9523.880010.14219.367029.962060.287IPMN IPMNDM2034.169513.76424.613551.563072.515PNET PNETNo DM2028.672013.87220.276062.8445433.460PNET PancreatitisDM1039.376513.96723.164044.9590111.390Pancreatitis PancreatitisNo DM7224.55106.45818.112330.4205209.117Pancreatitis Adenocarcinoma Stage I/II Adenocarcinoma Stage II/IVNo DM10235.049011.59025.583057.644024.8952Adenocarcinoma Stage III/IV DM2843.798315.69823.899065.134023.6424	Adenocarcinoma Stage III/IV	Female	60	40.5035	15.698	26.2460	67.0990	218.449
ControlsMale 12918.12106.42515.479022.327073.392IPMNNo DM9523.880010.14219.367029.962060.287IPMNDM2034.169513.76424.613551.563072.515PNETNo DM2028.672013.87220.276062.8445433.460PNETDM1039.376513.96723.164044.9590111.390PancreatitisNo DM7224.55106.45818.112330.4205209.117PancreatitisDM1026.171511.63718.447042.588077.424Adenocarcinoma Stage I/IINo DM10235.049011.59025.583057.6440248.952Adenocarcinoma Stage II/IVNo DM10838.771511.69927.787568.5970269.711Adenocarcinoma Stage III/IVDM2843.798315.69823.899065.1340238.642	Adenocarcinoma Stage III/IV	Male	76	40.8955	11.699	27.1895	67.2140	269.711
IPMN         No DM         95         23.8800         10.142         19.3670         29.9620         60.287           IPMN         DM         20         34.1695         13.764         24.6135         51.5630         72.515           PNET         No DM         20         28.6720         13.872         20.2760         62.8445         433.460           PNET         DM         10         39.3765         13.967         23.1640         44.9590         111.390           PAncreatitis         No DM         72         24.5510         6.458         18.1123         30.4205         209.117           Pancreatitis         DM         10         26.1715         11.637         18.4470         42.5880         77.424           Adenocarcinoma Stage I/II         No DM         102         35.0490         11.590         25.5830         57.6440         248.952           Adenocarcinoma Stage III/IV         No DM         108         38.7715         11.699         27.7875         68.5970         269.711           Adenocarcinoma Stage III/IV         DM         28         43.7983         15.698         23.8990         65.1340         238.642	Controls	Female	101	20.7900	12.012	17.2330	26.2680	65.573
IPMN         DM         20         34.1695         13.764         24.6135         51.5630         72.515           PNET         No DM         20         28.6720         13.872         20.2760         62.8445         433.460           PNET         DM         10         39.3765         13.967         23.1640         44.9590         111.390           Pancreatitis         No DM         72         24.5510         6.458         18.1123         30.4205         209.117           Pancreatitis         DM         10         26.1715         11.637         18.4470         42.5880         77.424           Adenocarcinoma Stage I/II         No DM         102         35.0490         11.590         25.5830         57.6440         248.952           Adenocarcinoma Stage II/IV         No DM         108         38.7715         15.599         23.2920         53.7950         451.397           Adenocarcinoma Stage III/IV         DM         28         43.7983         15.698         23.8990         65.1340         238.642	Controls	Male	129	18.1210	6.425	15.4790	22.3270	73.392
IPMN         DM         20         34.1695         13.764         24.6135         51.5630         72.515           PNET         No DM         20         28.6720         13.872         20.2760         62.8445         433.460           PNET         DM         10         39.3765         13.967         23.1640         44.9590         111.390           Pancreatitis         No DM         72         24.5510         6.458         18.1123         30.4205         209.117           Pancreatitis         DM         10         26.1715         11.637         18.4470         42.5880         77.424           Adenocarcinoma Stage I/II         No DM         102         35.0490         11.590         25.5830         57.6440         248.952           Adenocarcinoma Stage II/IV         No DM         108         38.7715         15.599         23.2920         53.7950         451.397           Adenocarcinoma Stage III/IV         DM         28         43.7983         15.698         23.8990         65.1340         238.642								
PNET         No DM         20         28.6720         13.872         20.2760         62.8445         433.460           PNET         DM         10         39.3765         13.967         23.1640         44.9590         111.390           Pancreatitis         No DM         72         24.5510         6.458         18.1123         30.4205         209.117           Pancreatitis         DM         10         26.1715         11.637         18.4470         42.5880         77.424           Adenocarcinoma Stage I/II         No DM         102         35.0490         11.590         25.5830         57.6440         248.952           Adenocarcinoma Stage II/IV         DM         50         37.2575         15.599         23.2920         53.7950         451.397           Adenocarcinoma Stage III/IV         DM         28         43.7983         15.698         23.8990         65.1340         238.642	IPMN	No DM	95	23.8800	10.142	19.3670	29.9620	60.287
PNET         DM         10         39.3765         13.967         23.1640         44.9590         111.390           Pancreatitis         No DM         72         24.5510         6.458         18.1123         30.4205         209.117           Pancreatitis         DM         10         26.1715         11.637         18.4470         42.5880         77.424           Adenocarcinoma Stage I/II         No DM         102         35.0490         11.590         25.5830         57.6440         248.952           Adenocarcinoma Stage II/IV         DM         50         37.2575         15.599         23.2920         53.7950         451.397           Adenocarcinoma Stage III/IV         No DM         108         38.7715         11.699         27.7875         68.5970         269.711           Adenocarcinoma Stage III/IV         DM         28         43.7983         15.698         23.8990         65.1340         238.642	IPMN	DM	20	34.1695	13.764	24.6135	51.5630	72.515
Pancreatitis Pancreatitis         No DM         72         24.5510         6.458         18.1123         30.4205         209.117           Pancreatitis         DM         10         26.1715         11.637         18.4470         42.5880         77.424           Adenocarcinoma Stage I/II Adenocarcinoma Stage III/IV         No DM         102         35.0490         11.590         25.5830         57.6440         248.952           No DM         50         37.2575         15.599         23.2920         53.7950         451.397           Adenocarcinoma Stage III/IV         No DM         108         38.7715         11.699         27.7875         68.5970         269.711           Adenocarcinoma Stage III/IV         DM         28         43.7983         15.698         23.8990         65.1340         238.642	PNET	No DM	20	28.6720	13.872	20.2760	62.8445	433.460
Pancreatitis         DM         10         26.1715         11.637         18.4470         42.5880         77.424           Adenocarcinoma Stage I/II         No DM         102         35.0490         11.590         25.5830         57.6440         248.952           Adenocarcinoma Stage I/II         DM         50         37.2575         15.599         23.2920         53.7950         451.397           Adenocarcinoma Stage III/IV         No DM         108         38.7715         11.699         27.7875         68.5970         269.711           Adenocarcinoma Stage III/IV         DM         28         43.7983         15.698         23.8990         65.1340         238.642	PNET	DM	10	39.3765	13.967	23.1640	44.9590	111.390
Adenocarcinoma Stage I/II         No DM 102         35.0490         11.590         25.5830         57.6440         248.952           Adenocarcinoma Stage I/II         DM 50         37.2575         15.599         23.2920         53.7950         451.397           Adenocarcinoma Stage III/IV         No DM 108         38.7715         11.699         27.7875         68.5970         269.711           Adenocarcinoma Stage III/IV         DM 28         43.7983         15.698         23.8990         65.1340         238.642	Pancreatitis	No DM	72	24.5510	6.458	18.1123	30.4205	209.117
Adenocarcinoma Stage I/II         DM         50         37.2575         15.599         23.2920         53.7950         451.397           Adenocarcinoma Stage III/IV         No DM         108         38.7715         11.699         27.7875         68.5970         269.711           Adenocarcinoma Stage III/IV         DM         28         43.7983         15.698         23.8990         65.1340         238.642	Pancreatitis	DM	10	26.1715	11.637	18.4470	42.5880	77.424
Adenocarcinoma Stage III/IV         No DM 108         38.7715         11.699         27.7875         68.5970         269.711           Adenocarcinoma Stage III/IV         DM 28         43.7983         15.698         23.8990         65.1340         238.642	Adenocarcinoma Stage I/II	No DM	102	35.0490	11.590	25.5830	57.6440	248.952
Adenocarcinoma Stage III/IV DM 28 43.7983 15.698 23.8990 65.1340 238.642	Adenocarcinoma Stage I/II	DM	50	37.2575	15.599	23.2920	53.7950	451.397
5	Adenocarcinoma Stage III/IV	No DM	108	38.7715	11.699	27.7875	68.5970	269.711
Controls No DM 201 19 2820 6 425 16 5060 23 6850 65 573	Adenocarcinoma Stage III/IV	DM	28	43.7983	15.698	23.8990	65.1340	238.642
	Controls	No DM	201	19.2820	6.425	16.5060	23.6850	65.573
Controls DM 29 19.3320 9.654 17.0210 24.4930 73.392	Controls	DM	29	19.3320	9.654	17.0210	24.4930	73.392

### Table S5B. Spearman correlation analysis of age and THBS2 values.

group	Spearman Correlation
IPMN	-0.00401
PNET	-0.15872
Pancreatitis	-0.02588
Adenocarcinoma Stage I/II	-0.08581
Adenocarcinoma Stage III/IV	-0.11019
Controls	0.06188

	Obstruc	tive J	laundice	(Tot	al Bilirubir	า ≥ 3.5)
	Unknown		No		Yes	Total
Stage	Ν	Ν	N %		%	Ν
I	0	4	80.0	1	20.0	5
IA	0	0	0.0	3	100.0	3
IB	0	8	80.0	2	20.0	10
II	2	42	80.77	10	19.23	52
IIA	1	16	57.14	12	42.86	28
IIB	2	36	73.47	13	26.53	49
111	3	35	71.43	14	28.57	49
IV	1	68	81.93	15	18.07	83
Total	9	:	209		70	279

#### Table S6A. Obstructive jaundice cases in the PDAC cohorts.

#### Table S6B. THBS2 and CA19-9 values and obstructive jaundice status.

Obstructive	Obstructive THBS2 (ng/ml)						
Jaundice	Ca19-9 (U/ml)	<42		≥ 42		Total	
		Ν	%	Ν	%	Ν	%
Unknown	< 55	1	100	0	0	1	1
Unknown	≥ 55	6	75	2	25	8	89
No	< 55	41	75	14	25	55	2
No	≥ 55	96	62	58	38	154	74
Yes	< 55	5	38	8	62	13	19
Yes	≥ 55	13	23	44	77	57	8,

## Table S6C. AUC values for CA19-9, THBS2, and combined markers by jaundice status in phases 2a and 2b of PDAC cases versus controls.

	-	Ca19-9		THBS2		CA19-9+THBS2	
	Ν	AUC	95% CI	AUC	95% CI	AUC	95% CI
Phase 2a PDAC	81/80	0.846	0.81, 0.88	0.843	0.80, 0.89	0.955	0.93, 0.98
Phase 2a PDAC - Jaundice	13/80	0.923	0.83, 1.00	0.931	0.87, 0.99	0.977	0.94, 1.00
Phase 2a PDAC – No Jaundice	65/80	0.831	0.78, 0.88	0.819	0.76, 0.88	0.950	0.92, 0.98
Phase 2b PDAC	197/140	0.881	0.86, 0.90	0.875	0.85, 0.90	0.970	0.96, 0.98
Phase 2b PDAC - Jaundice	54/140	0.891	0.85, 0.94	0.974	0.96, 0.99	0.991	0.98, 1.00
Phase 2b PDAC – No Jaundice	138/140	0.873	0.84, 0.90	0.837	0.80, 0.87	0.962	0.95, 0.98

Table S7A. Cross-tabulation of normal versus elevated THBS2 values, given a cutoff of 42 ng/ml, for the original cross-validation THBS2 assays ( $\kappa = 0.786$ ).

	TH	THBS2 Validation			
THBS2					
(ng/ml)	< 42	≥ 42	Total		
< 42	20	0	20		
≥ 42	4	14	18		
Total	24	14	38		

Table S7B. Cross-tabulation of normal versus elevated scaled THBS2 values, given a cutoff of 2.47, for the original and cross-validation THBS2 assays ( $\kappa = 0.895$ ).

	THBS2 Validation			
THBS2				
(ng/ml)	< 2.47	≥ 2.47	Total	
< 2.47	18	2	20	
≥ 2.47	0	18	18	
Total	18	20	38	

## Table S8. Summary of THBS2 immunohistochemistry in a total of 42 human PDAC and 4 cases of incidental PanIN and intraductal papillary mucinous neoplasm by immunohistochemistry.

	PDAC	PanINs/IPMN	% THBS2 positive in PDAC	% THBS2 positive in PanINs/IPMN
total (N)	42 (tissue array n=38, FCCC n= 4)	4 (FCCC)	100	100
THBS2 positive sections	42 (tissue array n=38, FCCC n=4)	4 (FCCC)	100	100
the number of sections THBS2 positive in epithelial cells of PDAC or PanINs/IPMN	32 (tissue array n=30, FCCC n=2)	4 (FCCC)	76	100
the number of sections THBS2 positive in both epithelial and fibroblast cells	21 (tissue array n=19, FCCC n=2)	0 (FCCC)	50	0
THBS2 positive in only poorly differentiated PDAC or fibroblast cells	8 (tissue array n=8, FCCC n=0)	0 (FCCC)	19	0

FCCC = samples from the repository at the Fox Chase Cancer Center