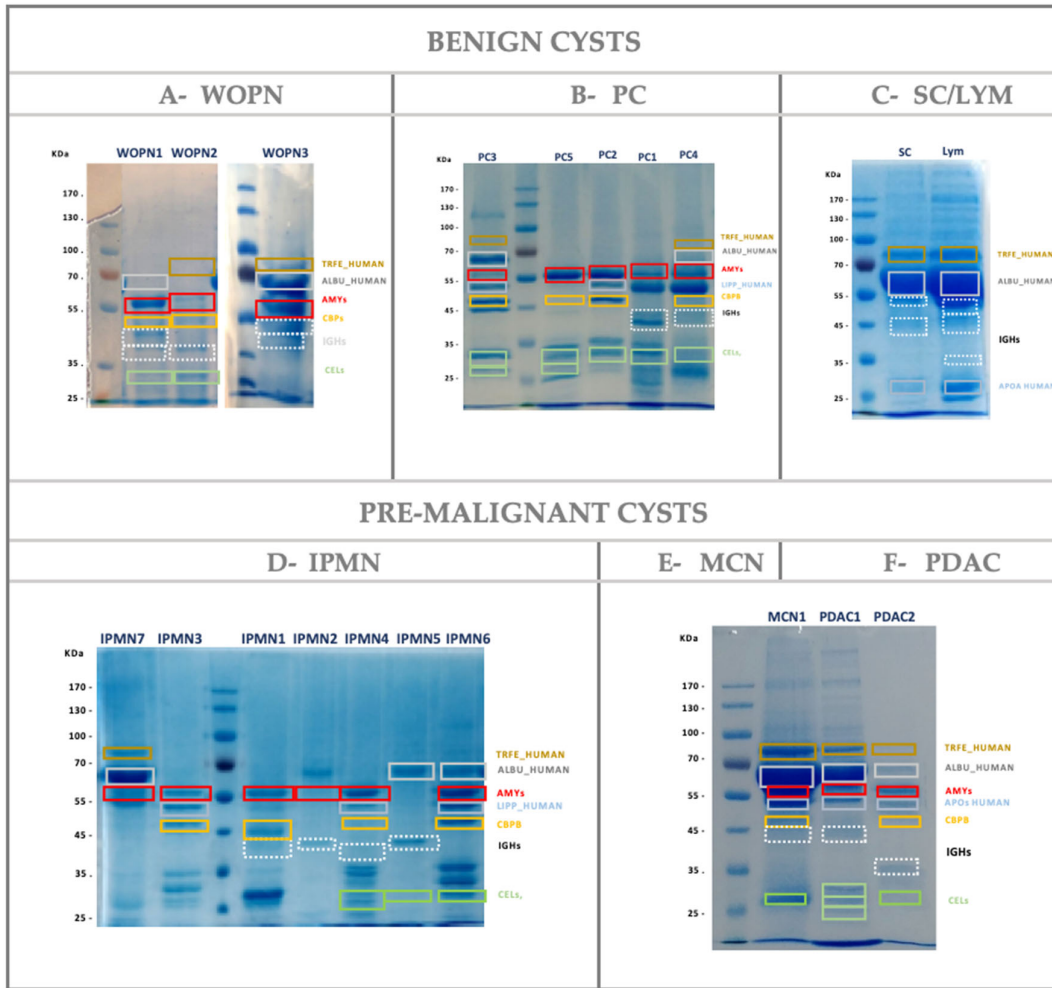


# Supplementary Materials: Thermal liquid biopsy (TLB) focused on benign and premalignant pancreatic cyst diagnosis

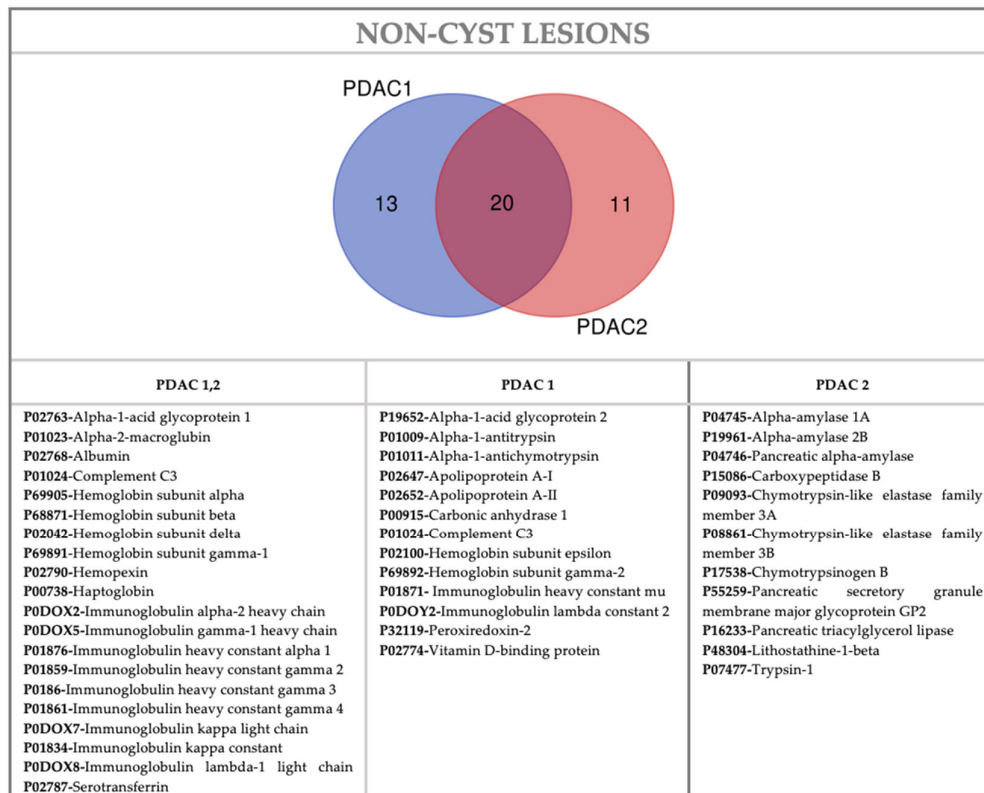
Sonia Hermoso-Durán, Guillermo García-Rayado, Laura Ceballos-Laita, Carlos Sostres, Sonia Vega, Judith Millastre, Oscar Sánchez-Gracia, Jorge L. Ojeda, Ángel Lanás, Adrián Velázquez-Campoy and Olga Abian

Category/Family	Accession Uniprot	Protein description	PC1	PC2	PC3	PC4	PC5	WOPN1	WOPN2	WOPN3	SC	LYM	IPMN1	IPMN2	IPMN3	IPMN4	IPMN6	IPMN7	MCN1	PDCA1	PDCA2			
Transport	P02768	Albumin																						
	Iron metabolism-related proteins	Hemoglobins	P68871	Hemoglobin subunit beta																				
			P69505	Hemoglobin subunit alpha																				
			P02042	Hemoglobin subunit delta																				
			P69891	Hemoglobin subunit gamma-1																				
			P68892	Hemoglobin subunit gamma-2																				
		P02100	Hemoglobin subunit epsilon																					
		Proteolytic proteins	P00738	Haptoglobin																				
			P02787	Serotransferrin																				
			P02788	Lactotransferrin																				
Transport-related proteins		P02792	Ferritin light chain																					
	P02790	Hemojuv																						
Oxidoreductases	P00450	Ceruloplasmin																						
	Macroglobulins	P01023	Alpha-2-macroglobulin																					
P01834		Immunoglobulin kappa constant																						
P000X5		Immunoglobulin gamma-1 heavy chain																						
P01876		Immunoglobulin heavy constant alpha 1																						
P01857		Immunoglobulin heavy constant gamma 1																						
P01859		Immunoglobulin heavy constant gamma 2																						
P01860		Immunoglobulin heavy constant gamma 3																						
P01861		Immunoglobulin heavy constant gamma 4																						
P000X8		Immunoglobulin lambda-1 light chain																						
P000Y2		Immunoglobulin lambda constant 2																						
P000X7		Immunoglobulin kappa light chain																						
P01871		Immunoglobulin heavy constant mu																						
P01833		Polymeric immunoglobulin receptor																						
P000X2	Immunoglobulin alpha-2 heavy chain																							
Other immune respons	P55259	Pancreatic secretory granule membrane major GP2																						
	P56665	Neutrophil defensin 1																						
Lipocalin family proteins	P02763	Alpha-1-acid glycoprotein 1																						
	P15652	Alpha-1-acid glycoprotein 2																						
	P01108	Neutrophil gelatinase-associated lipocalin																						
Complement related??	P01024	Complement C3																						
	P000L4	Complement C4-A																						
	P02774	Vitamin D-binding protein																						
	P04004	Vitronectin																						
Glycoside Hydrolases	P04746	Pancreatic alpha-amylase																						
	P04745	Alpha-amylase 1A																						
	P19961	Alpha-amylase 2B																						
	P61626	Lysozyme C																						
Proteolytic proteins	Metalloproteases	P15085	Carboxypeptidase A1																					
		P48052	Carboxypeptidase A2																					
		P15086	Carboxypeptidase B																					
	Elastases	P08098	Chymotrypsin-like elastase family member 3A																					
		P08217	Chymotrypsin-like elastase family member 2A																					
		Q98985	Chymotrypsin-C																					
		P08861	Chymotrypsin-like elastase family member 3B																					
		P07477	Trypsin-1																					
		P17538	Chymotrypsinogen B																					
	Mucines	P00995	Serine protease inhibitor Kazal-type 1																					
		P98086	Mucin 5AC																					
		Q9H434	Mucin 5B																					
		Q0W493	Mucin-6																					
		P20142	Gastricin																					
		P01009	Alpha-1-antitrypsin																					
	Lipid metabolism-related proteins	Proteolytic proteins	P01011	Alpha-1-antichymotrypsin																				
			Q14624	Inter-alpha-trypsin inhibitor heavy chain H4																				
			P15825	Bile salt-activated lipase																				
Lipases		P16233	Pancreatic triacylglycerol lipase																					
		P04118	Colipase																					
		P54317	Pancreatic lipase-related protein 2																					
		P04054	Phospholipase A2																					
		P02749	Beta-2-glycoprotein 1																					
		P04114	Apolipoprotein B-100																					
		P02647	Apolipoprotein A-I																					
Lipoproteins	P02652	Apolipoprotein A-II																						
	P06727	Apolipoprotein A-IV																						
	P02655	Apolipoprotein C-II																						
	P02656	Apolipoprotein C-III																						
	P06702	Protein S100-A9																						
S-100 family proteins	P05109	Protein S100-A8																						
	Coagulation	P02675	Fibrinogen beta chain																					
P02679		Fibrinogen gamma chain																						
Cell adhesion related-proteins	Q08380	Galectin-3-binding protein																						
	Q8LX06	Diffractomedin-4																						
Regeneration protein family	P05451	Lithostathine-3-alpha																						
	P48304	Lithostathine-3-beta																						
Others	P04217	Alpha-1B-glycoprotein																						
	P00015	Carbonic anhydrase 1																						
	P00918	Carbonic anhydrase 2																						
	P05164	Myeloperoxidase																						
P32119	Paraoxonase-2																							

Table S1: Detailed information of cyst proteomic profiles



**Figure S1:** Electrophoresis analysis of proteomic profiles

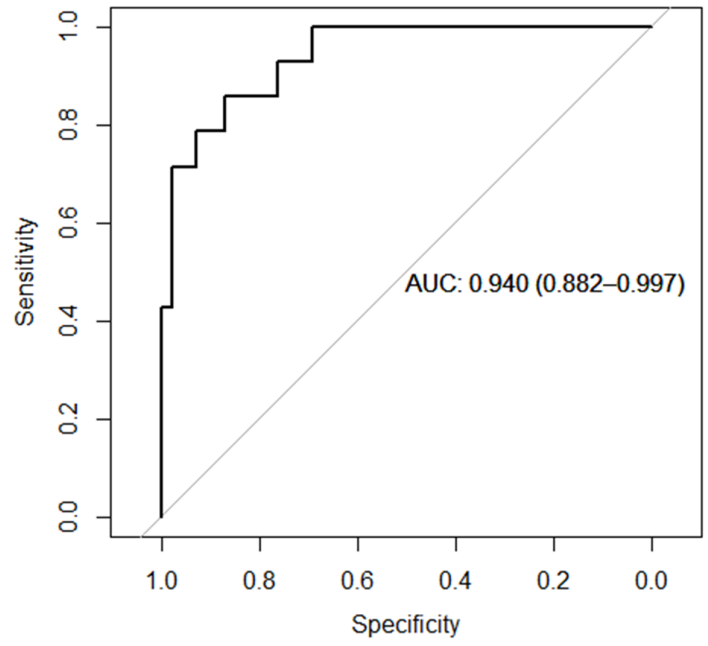


**Figure S2:** LC-ESI-MS/MS proteomic content of cyst samples types identification. Common proteins were analyzed via Venn diagrams online tool (<http://bioinformatics.psb.ugent.be/beg/tools/venn-diagrams>). The different colors represent different groups and numbers inside each set and shared subset indicate the number of identified proteins.

**Table S2:** Monovariant analysis of TLB parameters of healthy controls and cysts patients (p-value from Wilcoxon test)

<b>Parameter</b>	<b>Healthy Controls (n = 84)</b>	<b>Pancreatic Cysts (n = 14)</b>	<b>p-value</b>
T <sub>av</sub>	67.96 [67.60;68.39]	68.83 [68.52;68.97]	<b>&lt;0.001</b>
G <sub>1</sub>	0.26 [0.13;0.35]	0.36 [0.14;0.41]	0.215
WP	7.67 [7.13;8.25]	9.51 [8.78;10.61]	<b>&lt;0.001</b>
AUC <sub>n2</sub>	27.70 [20.08;45.60]	42.16 [29.97;56.02]	<b>0.047</b>
AP <sub>n2</sub>	1.19 [0.63;2.99]	1.93 [0.99;4.88]	0.247
AUC <sub>n3</sub>	22.80 [20.36;26.06]	24.10 [21.68;30.72]	0.141
AP <sub>n3</sub>	0.80 [0.58;1.01]	0.79 [0.67;1.09]	0.383
AUC <sub>n4</sub>	39.64 [28.86;52.37]	32.40 [22.40;44.53]	0.161
AP <sub>n4</sub>	2.42 [1.43;4.03]	1.40 [0.82;2.36]	0.076
AUC <sub>n5</sub>	43.06 [39.09;50.85]	44.44 [36.05;86.49]	0.707
AP <sub>n5</sub>	2.87 [2.25;3.82]	2.85 [1.80;9.51]	0.895
Dv <sub>2</sub>	1.19 [1.06;2.05]	0.89 [0.69;3.50]	0.084
Dv <sub>3</sub>	1.04 [1.01;1.12]	0.24 [0.15;0.51]	<b>&lt;0.001</b>
Dv <sub>4</sub>	1.18 [1.04;1.38]	0.63 [0.38;0.89]	<b>&lt;0.001</b>
Dv <sub>5</sub>	1.04 [1.00;1.17]	0.64 [0.31;2.51]	<b>0.020</b>

**Note:** median [Q1;Q3]



**Figure S3:** ROC curve illustrating the statistical performance of TLB serum score (healthy vs. cysts). AUC = Area Under Curve (95%CI)

**Table S3:** Monovariant analysis of TLB parameters of benign and premalignant cysts patients (p-value from t-test or Wilcoxon test depending on the normality character of parameter distribution)

Parameter	Benigno (n = 6)	Premaligno (n = 8)	p-value
T <sub>av</sub>	68.89 [68.85;69.22]	68.56 [68.41;68.85]	0.071
G <sub>1</sub>	0.18 (0.29)	0.32 (0.23)	0.349
WP	10.40 [9.50;11.47]	8.87 [8.58;9.84]	0.0939
AUC <sub>n2</sub>	37.77 (12.53)	48.53 (18.43)	0.2189
AP <sub>n2</sub>	1.06 [0.79;2.07]	3.33 [1.78;5.77]	0.1219
AUC <sub>n3</sub>	26.08 [23.83;30.72]	21.92 [20.60;27.85]	0.156
AP <sub>n3</sub>	0.96 [0.82;1.09]	0.71 [0.64;0.94]	0.156
AUC <sub>n4</sub>	41.98 [27.79;47.87]	30.69 [21.51;33.57]	0.366
AP <sub>n4</sub>	2.19 [1.20;3.08]	1.16 [0.74;1.64]	0.366
AUC <sub>n5</sub>	38.69 [34.40;58.27]	49.65 [40.10;97.86]	0.439
AP <sub>n5</sub>	2.29 [1.45;4.82]	3.21 [2.44;13.21]	0.366
Dv <sub>2</sub>	0.63 [0.22;1.22]	2.08 [0.75;4.35]	0.156
Dv <sub>3</sub>	0.27 [0.14;0.50]	0.23 [0.16;0.51]	1.000
Dv <sub>4</sub>	0.55 [0.29;0.98]	0.70 [0.50;0.86]	0.606
Dv <sub>5</sub>	0.69 [0.48;0.97]	0.53 [0.26;3.82]	0.897

**Note:** average (standard deviation) and median [Q1;Q3] are provided depending on the normality character of the parameter distribution.