

**Supplementary Material for:**

**A microRNA signature in circulating exosomes is superior to exosomal glypican-1 levels for diagnosing pancreatic cancer**

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**Supplemental Table 1.** Selectivity of GPC1 and the internal standard (IS) at non-spiking and low QC levels in 1% BSA

QC levels	Statistics	GPC1	IS	
Non-spiking (%)		1	30.3	0.4
		2	24.5	2.6
		3	24.2	0.8
		4	29.4	1.2
		5	25.8	3.0
		6	14.9	1.9
		Overall	24.8	1.7
Low	n	6	6	
	RSD (%)	0.0	35.2	
	Accuracy (%)	100.0	NA	

Supplemental Table 2. Clinical information and CA 19-9 levels

ID	Age	Gender	CA19-9 pre	CA19-9 post	Stage	Grade
PDAC 1	69	M	1390	900	III	mod poor
PDAC 2	79	F	1980	775	IIB	mod
PDAC 3	86	F	1355	1063	IIB	poor
PDAC 4	84	F	126	100	IIB	mod
PDAC 5	71	M	979	504	IIB	well mod
PDAC 6	50	M	241	14	IIB	mod
PDAC 7*	49	F	33	36	IIB	mod
PDAC 8	49	M	1230	1024	IIB	mod
PDAC 9	64	M	57	37	IIB	mod
PDAC 10	67	M	482	160	IIB	mod poor
PDAC 11	56	F	651	436	IIB	mod
PDAC 12*	70	M	45	17	IIB	poor
PDAC 13	67	M	63	55	IIB	poor
PDAC 14	61	M	128	49	IIB	mod
PDAC 15	70	M	635	397	IIB	mod
PDAC 16*	85	F	2	2	IIB	mod
PDAC 17	78	F	1586	1314	IV	mod
PDAC 18*	63	F	16	13	IIA	mod poor
PDAC 19	80	F	1919	604	IIB	mod
PDAC 20	50	M	406	195	IIB	poor
PDAC 21	90	F	152	33	IIB	mod
PDAC 22	67	M	428	340	IIB	mod
PDAC 23*	65	M	11	11	IIB	mod
PDAC 24*	73	M	17	16	IIB	mod
PDAC 25*	59	F	35	27	IA	mod
PDAC 26*	64	F	27	20	IIB	mod
PDAC 27	60	M	1093	366	IIB	poor
PDAC 28	56	F	153	76	IIB	mod
PDAC 29	71	F	114	57	IIB	mod

Plasma CA19-9 was measured in duplicate for each patient before and 24 hours post tumor resection. \*Denotes patients with low or only slightly elevated CA 19-9 levels.

Supplemental Table 3. Exosomal miR levels in 29 PDAC cases

Exosomes	miR-10b	miR-21	miR-30c	miR-106b	let7a	miR-20a	miR-122	miR-181a	miR-483
PDAC 1	12.12	5.40	11.14	1.06	0.33	2.34	0.31	7.12	1.22
PDAC 2	32.77	2.70	15.14	2.46	0.10	4.11	0.21	11.40	3.50
PDAC 3	15.80	17.10	13.75	0.82	0.46	1.61	0.37	3.68	0.63
PDAC 4	14.91	8.04	14.05	1.09	0.38	0.87	0.48	22.47	0.24
PDAC 5	26.32	4.25	13.21	1.97	0.61	1.99	0.36	12.33	2.22
PDAC 6	92.00	11.18	12.68	1.40	0.39	1.86	0.65	9.91	4.24
PDAC 7	7.88	4.60	4.17	1.64	0.28	3.13	0.40	7.07	2.30
PDAC 8	10.47	4.28	8.81	1.32	0.40	2.90	0.50	5.91	5.90
PDAC 9	34.61	12.28	22.10	1.52	0.18	2.46	0.58	8.21	0.12
PDAC 10	18.73	4.15	20.30	1.56	0.51	2.07	0.35	2.99	2.94
PDAC 11	17.58	5.37	14.15	2.44	0.51	2.50	0.19	7.31	3.62
PDAC 12	19.40	4.39	13.36	2.76	0.28	2.86	0.22	11.61	5.15
PDAC 13	22.19	2.87	11.16	0.62	0.55	3.96	0.20	4.34	0.96
PDAC 14	12.95	45.54	99.76	16.27	0.34	2.65	0.49	15.64	0.88
PDAC 15	14.16	3.39	49.00	1.64	0.54	3.33	0.45	4.44	0.17
PDAC 16	129.39	6.92	171.88	10.78	0.33	2.46	0.79	6.88	0.14
PDAC 17	123.10	9.69	102.35	13.98	0.71	4.81	0.70	12.06	3.82
PDAC 18	20.46	14.15	67.82	4.19	0.29	1.46	0.35	19.02	1.46
PDAC 19	77.02	8.49	40.55	8.21	0.53	1.35	0.83	12.34	1.85
PDAC 20	66.74	14.03	46.30	3.31	0.44	1.83	0.27	6.75	1.73
PDAC 21	31.63	6.30	21.41	2.54	0.11	2.65	0.69	9.41	0.79
PDAC 22	28.47	4.63	30.00	4.82	0.56	1.23	0.61	6.21	0.40
PDAC 23	54.19	1.40	22.78	9.01	0.25	1.99	0.30	8.14	4.17
PDAC 24	17.36	6.76	7.69	1.97	0.40	2.48	0.33	3.78	0.35
PDAC 25	14.40	4.19	4.88	2.76	0.10	2.17	0.04	1.44	0.89
PDAC 26	27.62	7.13	14.40	5.96	0.22	2.12	0.04	4.90	0.81
PDAC 27	9.86	1.79	3.43	0.71	0.63	3.10	0.16	7.11	0.46
PDAC 28	83.35	4.15	8.54	2.36	0.50	3.58	0.55	8.50	2.64
PDAC 29	29.86	8.13	14.30	2.88	0.67	4.47	0.14	5.40	0.14

Supplemental Table 4. Plasma miR levels in 29 PDAC cases

Plasma	miR-10b	miR-21	miR-30c	miR-106b	let7a	miR-20a	miR-122	miR-181a	miR-483
PDAC 1	19.28	7.35	7.34	4.75	0.29	4.22	0.71	2.87	1.03
PDAC 2	13.64	10.94	8.57	12.53	0.51	8.11	0.28	2.37	2.10
PDAC 3	11.23	4.63	8.72	5.71	0.40	7.10	0.31	3.81	1.26
PDAC 4	9.34	13.67	4.01	3.79	0.98	12.43	0.42	2.57	0.12
PDAC 5	9.60	5.93	14.55	6.29	0.76	3.89	0.90	3.25	1.94
PDAC 6	40.66	13.87	15.62	3.34	0.61	13.60	0.56	1.64	1.63
PDAC 7	10.90	7.20	15.55	7.15	0.87	7.32	0.56	1.25	3.51
PDAC 8	27.65	6.22	7.46	9.35	0.86	8.71	0.51	1.77	1.72
PDAC 9	12.83	5.21	9.78	9.27	0.48	9.54	0.41	1.47	0.35
PDAC 10	21.59	5.65	24.67	5.21	0.55	6.72	0.44	8.27	2.44
PDAC 11	10.07	8.61	8.22	3.38	0.45	4.82	0.70	5.90	1.70
PDAC 12	14.81	3.99	9.66	1.60	0.76	5.55	0.82	1.39	1.63
PDAC 13	14.99	6.69	8.58	3.18	0.70	1.94	0.55	1.39	4.74
PDAC 14	5.64	3.90	4.99	4.37	0.53	4.29	0.92	1.25	0.98
PDAC 15	6.29	1.22	7.49	2.39	0.15	8.66	0.43	3.15	3.19
PDAC 16	7.90	4.19	42.70	12.90	0.73	1.99	0.22	2.77	1.40
PDAC 17	15.21	1.27	16.40	6.09	0.26	8.89	0.48	5.01	0.80
PDAC 18	8.73	6.03	10.12	3.32	0.83	3.86	0.33	2.27	3.14
PDAC 19	9.60	2.86	3.85	4.21	0.77	7.84	0.64	7.29	1.01
PDAC 20	16.06	4.03	1.57	5.29	0.55	3.86	0.66	2.99	4.14
PDAC 21	4.44	3.17	4.68	6.11	0.43	2.92	0.11	3.27	0.36
PDAC 22	17.66	4.06	7.77	13.20	0.63	10.46	0.73	3.41	3.74
PDAC 23	9.06	3.32	11.61	3.86	0.38	5.38	0.59	3.96	3.56
PDAC 24	15.90	4.10	5.81	1.22	0.40	2.82	0.38	0.54	3.51
PDAC 25	5.64	1.71	7.50	0.67	0.13	3.95	0.49	1.69	1.55
PDAC 26	5.80	1.24	4.11	1.55	0.58	4.79	0.31	1.30	8.35
PDAC 27	12.76	12.21	3.39	5.37	0.38	5.88	0.62	4.44	1.99
PDAC 28	12.47	0.94	10.48	3.63	0.80	7.72	0.36	3.81	0.36
PDAC 29	9.94	2.74	3.91	4.12	0.39	4.22	0.60	2.19	1.84

## Supplementary Figure Legends

### **Supplementary Fig. S1. Size distribution of exosomes from control subjects.**

Exosomes from the plasma of three control subjects were assessed for size using the qNano capture protocol and NP100 nanopore. Data were graphed as the percent of the population versus size, with controlled binning parameters utilizing the Data Analysis feature in the Izon Control Suite 3.2.

**Supplementary Fig. S2A. Size distribution of exosomes from chronic pancreatitis patients prior to surgery.** Exosomes isolated from the plasma of three chronic pancreatitis patients prior to pancreatectomy were assessed for size as described for Fig. S1.

**Supplementary Fig. S2B. Size distribution of exosomes from chronic pancreatitis patients following pancreatectomy.** Exosomes were isolated from the plasma of three chronic pancreatitis patients 24 hr following pancreatectomy, and assessed for size as described for Fig. S1.

**Supplementary Fig. S3A. Size distribution of exosomes from PDAC patients prior to surgery.** Exosomes were prepared from the plasma of patients with PDAC prior to tumor resection, and assessed for size as described for Fig. S1.

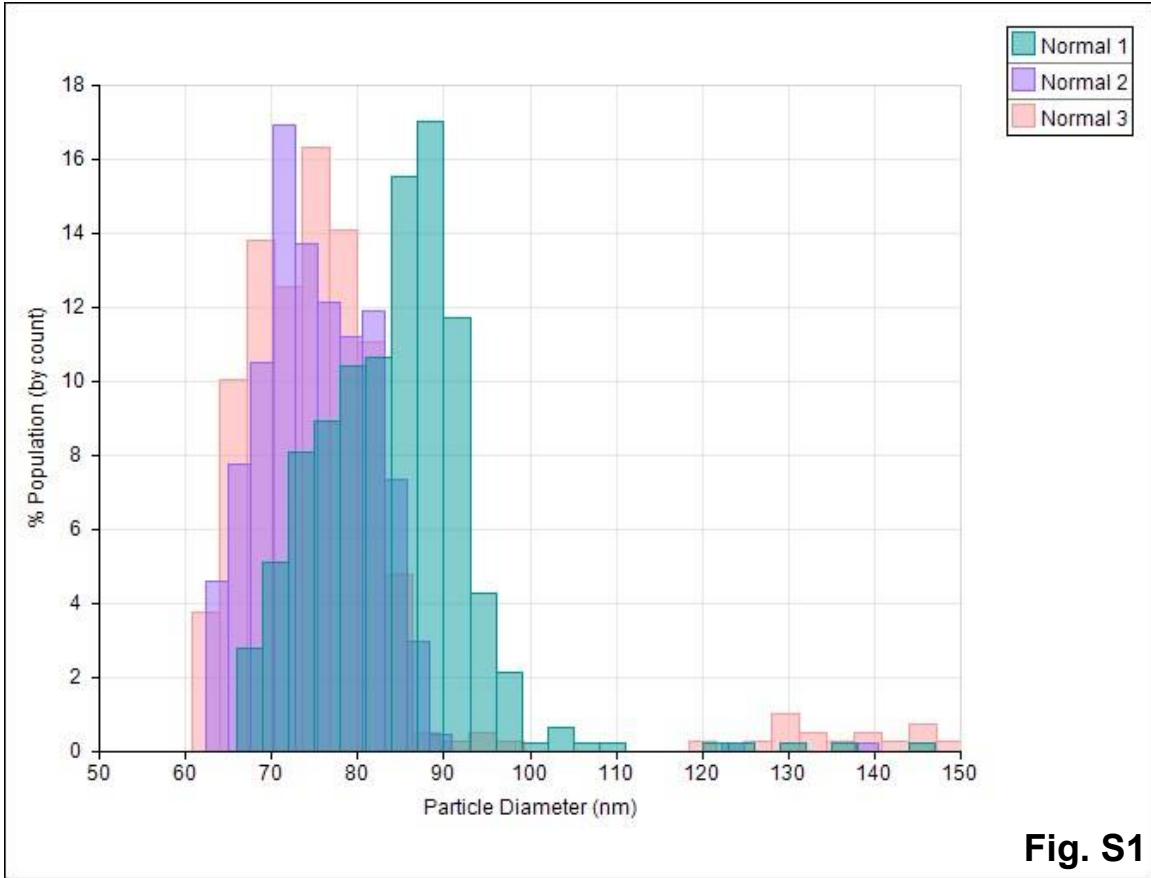
**Supplementary Fig. S3B. Size distribution of exosomes from PDAC patients following surgery.** Exosomes were prepared from the plasma of patients with PDAC following resection, and assessed for size as described for Fig. S1.

**Supplementary Fig. S4. Purity of exosome preparation.** Exosomes were isolated by ultracentrifugation from the plasma of a patient with PDAC. Pellets were suspended in 2% glutaraldehyde/2% paraformaldehyde. A 5  $\mu$ l suspension was placed on top of a Formvar-coated EM grid. Exosomes were then negatively stained with 2.0% uranyl acetate and imaged in the Electron Microscopy Center using a Tecnai G2 12 Bio Twin TEM microscope and AMT CCD camera.

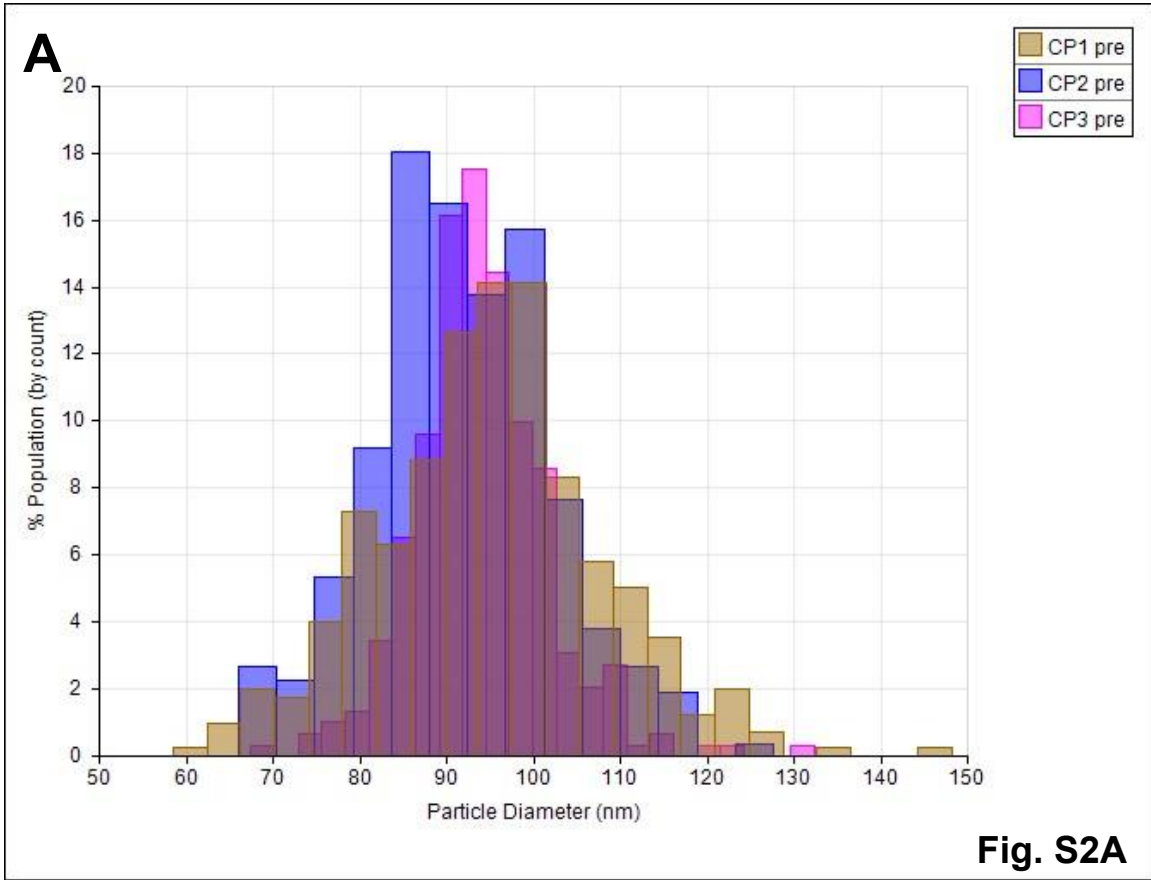
**Supplementary Fig. S5.** Immunoblot of exosome lysates. Exosomes were isolated from the plasma of three control subjects, and from three pre- and post-surgery (Sx) patients with CP and PDAC. Exosomal protein lysates were probed for CD63.

**Supplementary Fig. S6.** Receiver operator characteristic (ROC) is based on the levels of glypican-1 (GPC1) in exosomes prior to surgery, derived by using the SigmaPlot 13.0 ROC Curves macro tool and comparing levels in PDAC v. normal controls. AUC: Area under the curve.

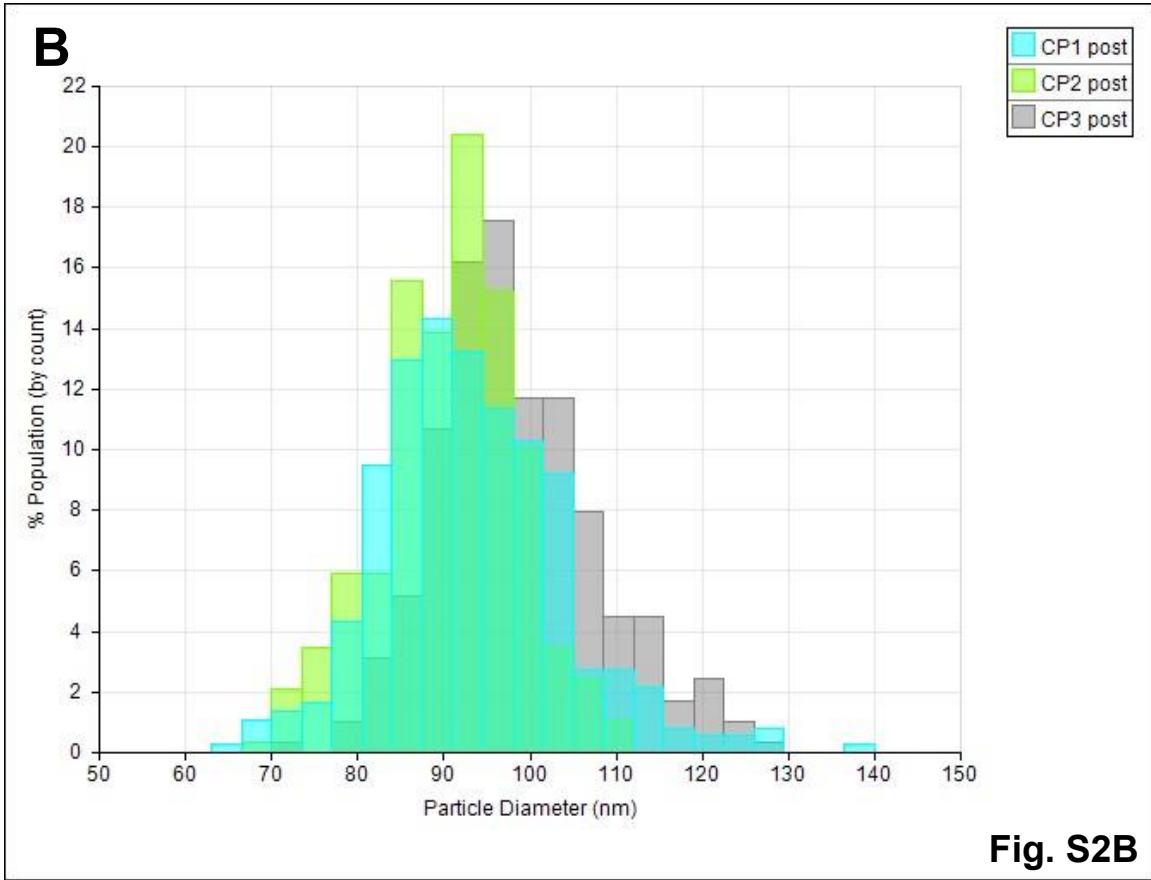
**Supplementary Fig. S7.** Receiver operator characteristic (ROC) is based on the levels of CA 19-9 in exosomes prior to surgery, derived by using the SigmaPlot 13.0 ROC Curves macro tool. AUC: Area under the curve.

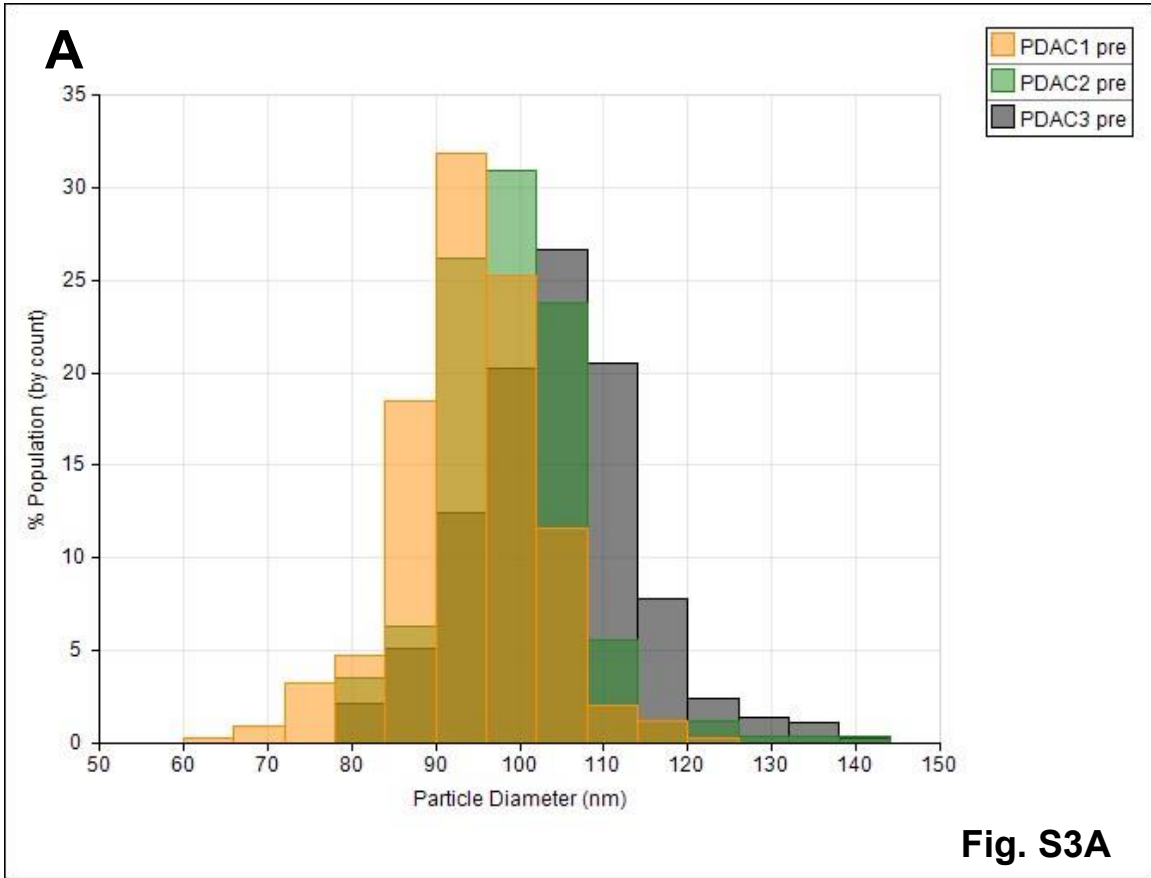


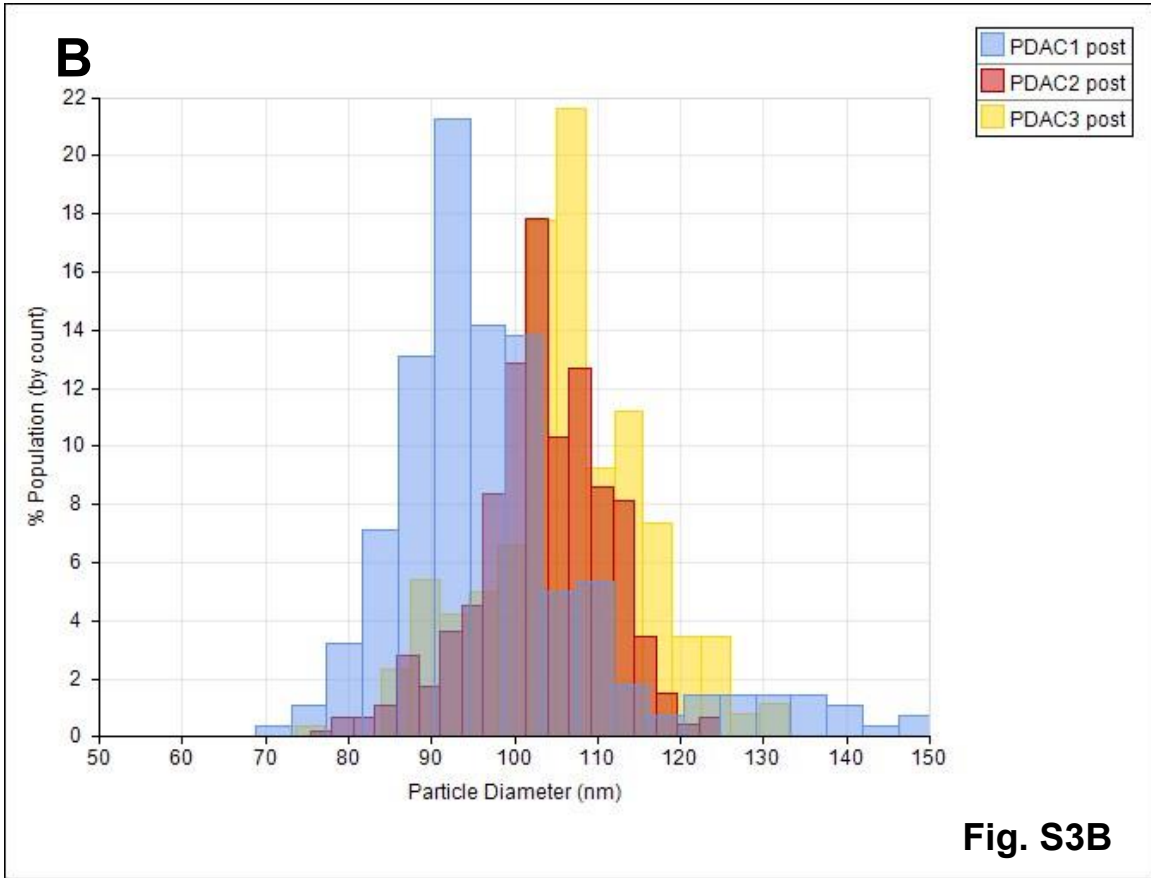
**Fig. S1**

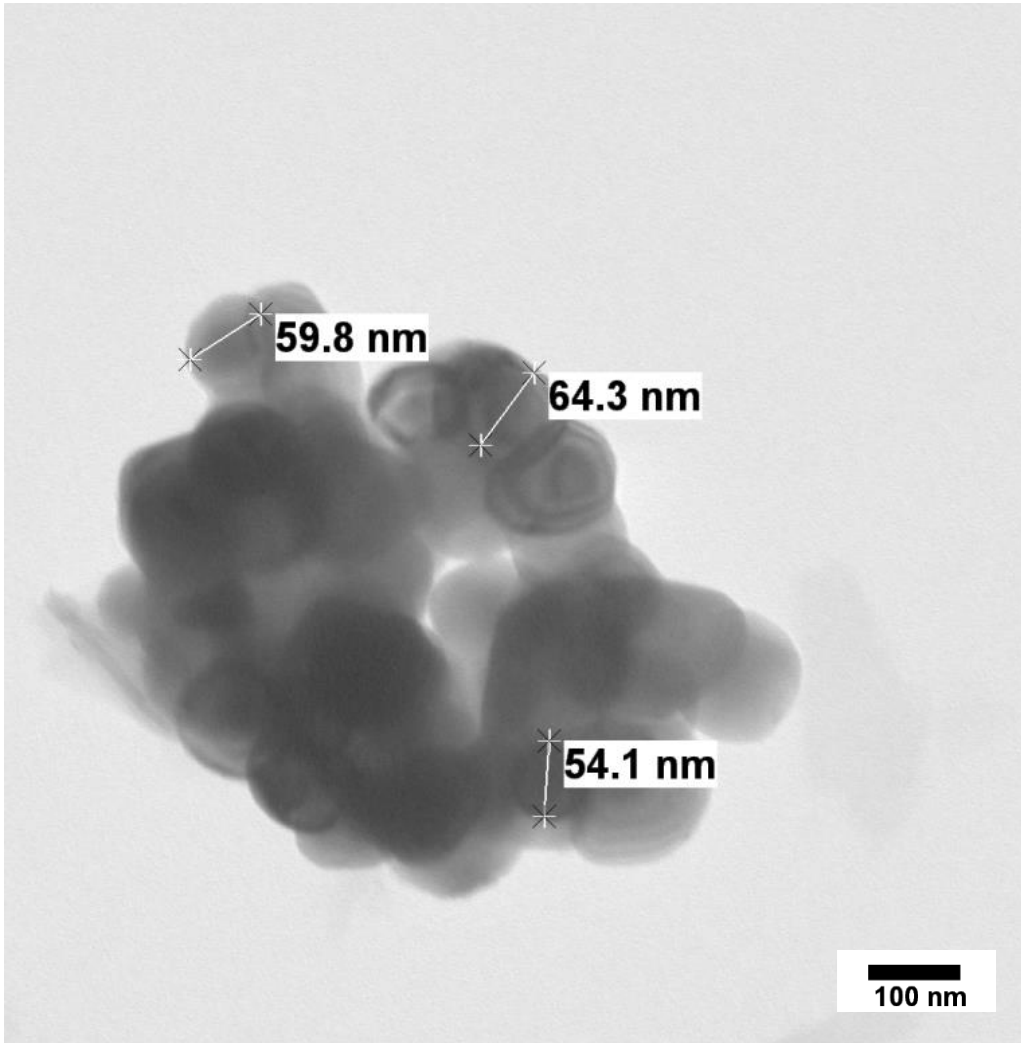












**Fig. S4**

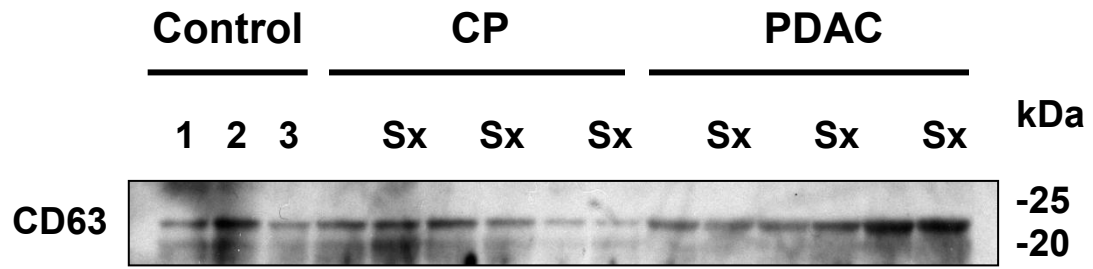


Fig. S5

# GPC1 LC/MS-MS PDAC

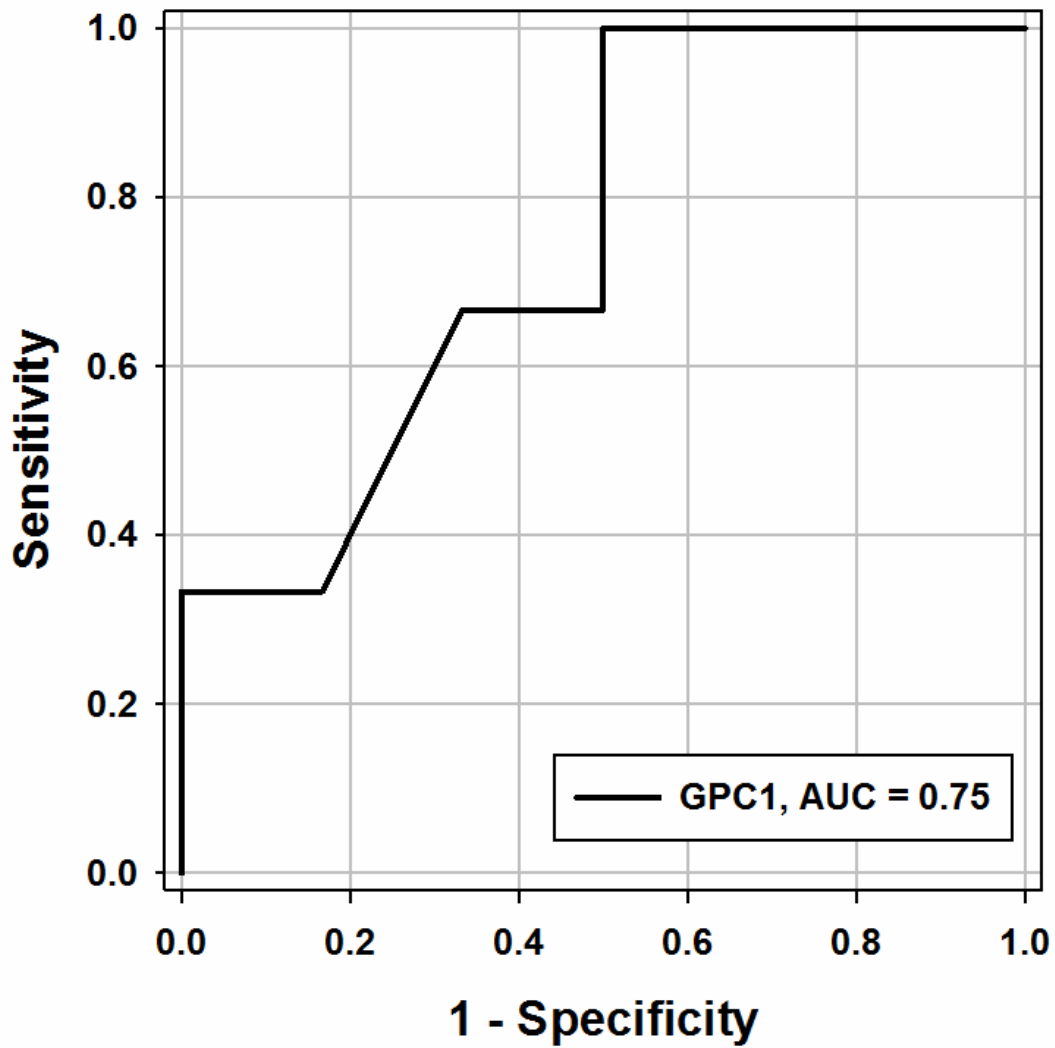


Fig. S6

# CA19-9 ROC Curve

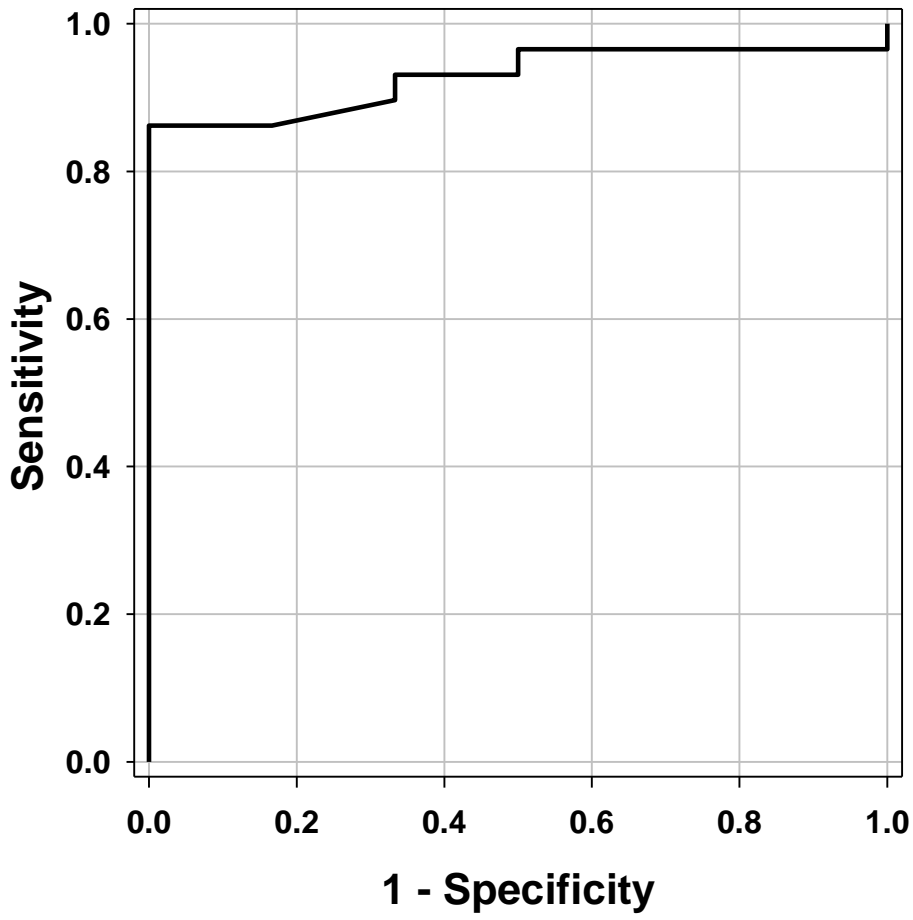


Fig. S7

	Specificity	Sensitivity	AUC	Cutoff	p-value
CA19-9	100%	86%	0.92	22.5	0.001