Supplementary Material

Case/non-case Group Requirements

Case requirements:

(1) Based on the diagnostic criteria of PCa and treatment specifications (2018 version);

(2) Subjects with first diagnosis or relapse of PCa;

(3) Before receiving surgical treatment, radiotherapy, and chemotherapy.

Non-case requirements

(1) Subjects of other pancreatic diseases, such as chronic pancreatitis, pancreatic cystadenoma, pancreatic pseudocyst, insulinoma, solid pseudopapilledema, etc.;

(2) Subjects with diabetes and obesity. Diabetes: random blood glucose >11.1 mmol/L, fasting blood glucose >7 mmol/L, or glucose load >11.1 mmol/L (post 2 hours); Obesity: BMI ≥ 28 Kg/m^2;

(3) Subjects with other digestive tract diseases, such as abdominal pain, jaundice, etc.;

(4) Subjects with other digestive tract/digestive system tumors, such as liver cancer, gastric cancer, esophageal cancer, bowel cancer, etc.

Inclusion and Exclusion Criteria

Inclusion criteria

(1) Aged ≥18 years old, gender unlimited;

(2) Serum samples from individuals who meet the requirements of the case group/non-case group;

(3) The minimum requirement of serum volume is 1 mL;

(4) Subjects met the hospital's informed consent process or voluntarily signed the informed consent form.

Exclusion criteria

(1) Samples with insufficient serum;

(2) Hemolytic sample;

(3) Storage, transportation, treatment, and other samples that do not meet the requirements;

(4) Multiple samples of the same subject;

(5) Other situations that the researcher believes need to be excluded.

Rejection criteria

(1) Incomplete case data (information);

(2) Detection failure due to unqualified sample quality, test operation, and other reasons;

(3) Other situations that the researcher believes need to be eliminated.

Supplementary Tables

Supplementary Table 1. Performance of the miRNA panel in discriminating patients with PDAC from non-PDAC subjects (CP/HC) in the training and validation cohorts

Scenario	Sensitivity (95%CI)	Specificity (95%CI)	Accuracy (95%CI)	AUC (95%CI)	True Positive	True Negative	False Positive	False Negative
Training cohort								
PDAC vs non-PDAC	83.5% (79.2%-87.8%)	84.0% (80.2%-87.8%)	83.8% (83.7%-83.8%)	0.920 (0.901-0.940)	238	294	56	47
PDAC vs CP	81.1% (76.5%-85.6%)	86.7% (76.7%-96.6%)	81.8% (81.7%-81.9%)	0.906 (0.859-0.954)	231	39	6	54
PDAC vs HC	95.1% (92.6%-97.6%)	88.8% (84.4%-93.2%)	92.5% (92.5%-92.6%)	0.970 (0.956-0.984)	271	175	22	14
Validation cohort								
PDAC vs non-PDAC	88.5% (84.8%-92.2%)	81.2% (77.2%-85.3%)	84.5% (84.4%-84.5%)	0.849 (0.810-0.887)	245	299	53	41
PDAC vs CP	83.9% (79.7%-88.2%)	82.2% (71.1%-93.4%)	83.7% (83.6%-83.8%)	0.831 (0.754-0.908)	240	37	8	46
PDAC vs HC	95.1% (92.6%-97.6%)	87.9% (83.3%-92.4%)	92.1% (92.1%-92.2%)	0.915 (0.880-0.950)	272	174	24	14

Supplementary Table 2. Performance of the miRNA panel tested in subjects with PDAC at stages (TNM) compared with that of HC

Stages (TNM)	**Subjects recruited**	**Subjects tested positive**	**Subjects tested negative**	**Specificity (95%CI)**	**Sensitivity (95%CI)**	**Accuracy (95%CI)**
Training cohort						
I	69	60	9	92.4% (88.7%-96.1%)	94.2% (88.7%-99.7%)	92.9% (92.8%-92.9%)
II	108	91	17	89.3% (85.0%-93.6%)	95.4% (91.4%-99.3%)	91.5% (91.4%-91.5%)
III	44	35	9	89.3% (85.0%-93.6%)	90.9% (82.4%-99.4%)	89.6% (89.6%-89.7%)
IV	30	25	5	90.9% (86.8%-94.9%)	93.3% (84.4%-92.9%)	91.2% (91.1%-91.3%)
Validation cohort						
I	60	54	6	92.4% (88.7%-96.1%)	93.3% (87.0%-99.6%)	92.6% (92.6%-92.7%)
II	117	103	14	88.4% (83.9%-92.8%)	96.6% (93.3%-99.9%)	91.4% (91.4%-91.5%)
III	48	36	12	89.9% (85.7%-94.1%)	91.7% (83.8%-99.5%)	90.2% (90.2%-90.3%)
IV	33	27	6	90.4% (86.3%-94.5%)	84.8% (72.6%-97.1%)	89.6% (89.5%-89.7%)

Supplementary Table 3. Performance of the miRNA panel and common biomarkers in discriminating patients with early-stage PDAC from non- PDAC subjects (CP/HC) in the training cohort

Scenario	Sensitivity（95%CI）	Specificity（95%CI）	Accuracy（95%CI）	AUC（95%CI）
miRNA panel				
Early-stage PDAC vs non-PDAC	93.8%（89.4%-98.2%）	75.7% (71.2%-80.2%)	80.1% (80.1%-80.2%)	0.924 (0.899-0.949)
Early-stage PDAC vs CP	81.4% (74.2%-88.6%)	86.7% (76.7%-96.6%)	82.9% (82.7%-83.1%)	0.905 (0.853-0.956)
Early-stage PDAC vs HC	92.9% (88.2%-97.6%)	92.9% (89.3%-96.5%)	92.9% (92.9%-92.9%)	0.971 (0.956-0.987)
CA125				
Early-stage PDAC vs non-PDAC	81.2% (73.4%-89.1%)	53.7% (47.6%-59.8%)	61.3% (61.1%-61.4%)	0.677 (0.622-0.732)
Early-stage PDAC vs CP	9.4% (3.5%-15.2%)	97.2% (91.9%-102.6%)	33.3% (33.0%-33.7%)	0.468 (0.358-0.578)
Early-stage PDAC vs HC	82.3% (74.7%-89.9%)	66.0% (58.4%-73.6%)	72.4% (72.2%-72.5%)	0.719 (0.655-0.783)
CA19-9				
Early-stage PDAC vs non-PDAC	69.5% (60.7%-78.3%)	80.6% (76.0%-85.1%)	77.6% (77.5%-77.7%)	0.790 (0.732-0.849)
Early-stage PDAC vs CP	52.4% (42.8%-61.9%)	94.7% (87.6%-101.8%)	63.6% (63.3%-64.0%)	0.688 (0.602-0.774)
Early-stage PDAC vs HC	82.9% (75.6%-90.1%)	75.9% (69.5%-82.3%)	78.5% (78.4%-78.7%)	0.820 (0.763-0.878)
CA242				
Early-stage PDAC vs non-PDAC	67.8% (55.9%-79.7%)	69.8% (63.8%-75.9%)	69.4% (69.2%-69.5%)	0.680 (0.598-0.762)
Early-stage PDAC vs CP	54.2% (41.5%-66.9%)	100.0% (100.0%-100.0%)	70.3% (69.9%-70.8%)	0.711 (0.606-0.816)
Early-stage PDAC vs HC	67.8% (55.9%-79.7%)	72.7% (65.5%-79.8%)	71.3% (71.1%-71.5%)	0.682 (0.600-0.765)
CEA				
Early-stage PDAC vs non-PDAC	82.7% (75.4%-90.0%)	41.2% (35.3%-47.1%)	52.8% (52.7%-53.0%)	0.580 (0.522-0.638)
Early-stage PDAC vs CP	13.5% (6.9%-20.0%)	97.4% (92.3%-102.5%)	35.9% (35.6%-36.2%)	0.432 (0.319-0.546)
Early-stage PDAC vs HC	98.1% (95.4%, 100.7%)	26.7% (19.6%, 33.7%)	55.9% (55.7%, 56.1%)	0.520 (0.448, 0.591)

Supplementary Table 4. Performance of the miRNA panel and common biomarkers in discriminating patients with early-stage PDAC from non-PDAC subjects (CP/HC) in the validation cohort

Scenario	Sensitivity（95%CI）	Specificity（95%CI）	Accuracy（95%CI）	AUC（95%CI）
miRNA panel				
Early-stage PDAC vs non-PDAC	95.1%(91.0%-99.3%)	77.0%(72.6%-81.4%)	81.1%(81.0%-81.2%)	0.861(0.818-0.903)
Early-stage PDAC vs CP	80.6%(72.9%-88.2%)	82.2%(71.1%-93.4%)	81.1%(80.9%-81.3%)	0.814(0.720-0.908)
Early-stage PDAC vs HC	94.2%(89.7%-98.7%)	92.4%(88.7%-96.1%)	93.0%(93.0%-93.1%)	0.933(0.892-0.974)
CA125				
Early-stage PDAC vs non-PDAC	65.5%(55.5%-75.5%)	58.6%(52.5%-64.8%)	60.4%(60.3%-60.6%)	0.621(0.540-0.701)
Early-stage PDAC vs CP	14.9%(7.5%-22.4%)	94.6%(87.3%-101.9%)	38.7%(38.3%-39.1%)	0.548(0.474-0.622)
Early-stage PDAC vs HC	69.0%(59.2%-78.7%)	72.9%(65.7%-80.2%)	71.4%(71.3%-71.6%)	0.709(0.625-0.794)
CA19-9				
Early-stage PDAC vs non-PDAC	63.5%(53.9%-73.2%)	63.5%(53.9%-73.2%)	78.7%(78.6%-78.8%)	0.737(0.668-0.807)
Early-stage PDAC vs CP	46.9%(36.9%-56.9%)	94.7%(87.6%-101.8%)	60.4%(60.1%-60.8%)	0.708(0.623-0.793)
Early-stage PDAC vs HC	79.2%(71.0%-87.3%)	83.7%(78.1%-89.3%)	82.1%(82.0%-82.2%)	0.815(0.746-0.883)
CA242				
Early-stage PDAC vs non-PDAC	78.1%(68.0%-88.3%)	66.4%(60.1%-72.6%)	69.0%(68.9%-69.2%)	0.722(0.641-0.804)
Early-stage PDAC vs CP	65.6%(54.0%-77.3%)	87.9%(76.7%-99.0%)	73.2%(72.8%-73.6%)	0.768(0.654-0.881)
Early-stage PDAC vs HC	78.1%(68.0%-88.3%)	70.8%(63.4%-78.3%)	73.1%(72.9%-73.3%)	0.745(0.657-0.833)
CEA				
Early-stage PDAC vs non-PDAC	77.7%(69.2%-86.1%)	38.1%(32.2%-44.1%)	48.7%(48.6%-48.9%)	0.579(0.507-0.651)
Early-stage PDAC vs CP	27.7%(18.6%-36.7%)	89.5%(79.7%-99.2%)	45.5%(45.1%-45.8%)	0.586(0.492-0.680)
Early-stage PDAC vs HC	96.8%(93.3%-100.4%)	15.3%(9.4%-21.2%)	47.5%(47.3%-47.7%)	0.560(0.513-0.608)

Supplementary Table 5. Performance of the miRNA panel in discriminating patients with pancreatic cystic neoplasm (PCN) from early-stage PDAC subjects (CP/HC) in the training and validation cohorts

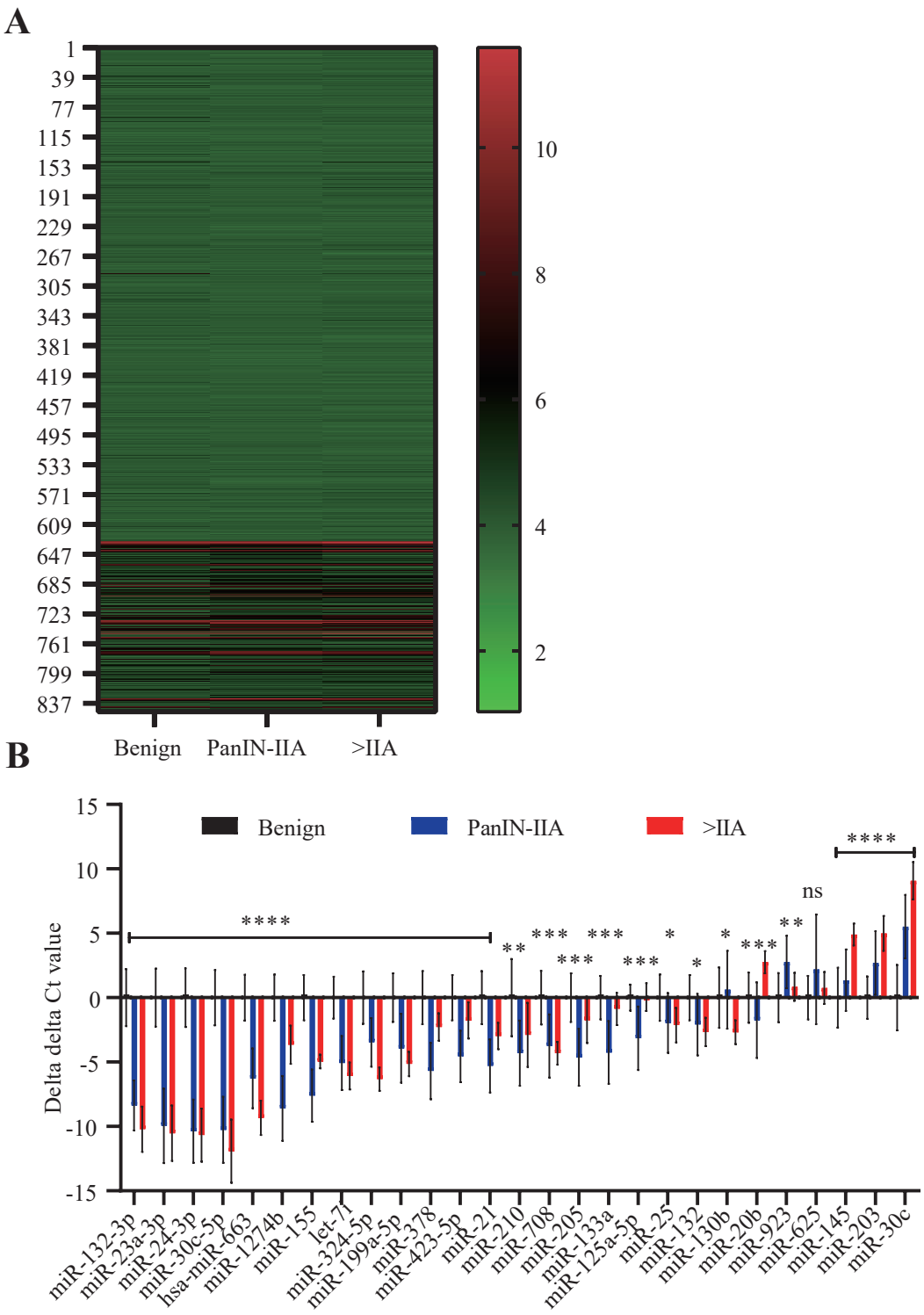
Scenario	Sensitivity (95%CI)	Specificity (95%CI)	Accuracy (95%CI)	AUC (95%CI)	True Positive	True Negative	False Positive	False Negative
Training cohort								
PCN vs CP	66.3% (56.1%-76.4%)	77.8% (65.6%-89.9%)	70.3% (70.0%-70.6%)	0.736 (0.645-0.827)	55	35	10	28
PCN vs HC	92.8% (87.2%-98.3%)	72.6% (66.4%-78.8%)	78.6% (78.5%-78.7%)	0.878 (0.834-0.922)	77	143	54	6
PCN vs Early-stage PDAC	84.3% (76.5%-92.2%)	68.1% (59.6%-76.7%)	75.0% (74.8%-75.2%)	0.836 (0.781-0.891)	70	77	36	13
Validation cohort								
PCN vs CP	57.1% (46.6%-67.7%)	68.9% (55.4%-82.4%)	61.2% (60.9%-61.6%)	0.630 (0.510-0.751)	48	31	14	36
PCN vs HC	90.5% (84.2%-96.8%)	69.7% (63.3%-76.1%)	75.9% (75.8%-76.0%)	0.801 (0.737-0.864)	76	138	60	8
PCN vs Early-stage PDAC	82.1% (74.0%-90.3%)	68.0% (58.9%-77.0%)	74.3% (74.1%-74.5%)	0.751 (0.665-0.837)	69	70	33	15

Supplementary Table 6. Univariate and multivariate logistic regression analysis of risk factors of PCa

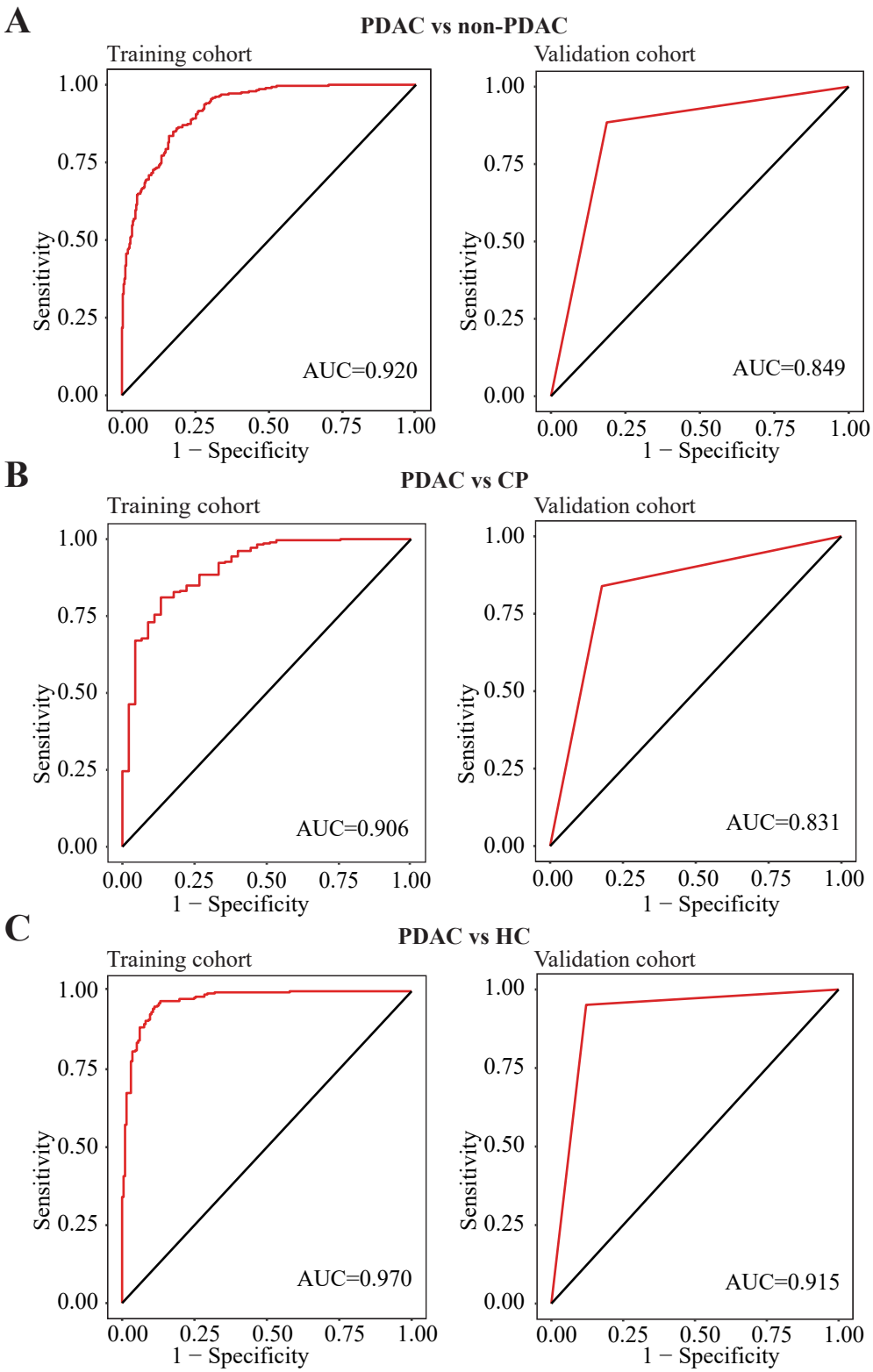
	Univariate analysis			Multivariate analysis		
Variables	**P Value**	**Estimate OR**	**95%CI**	**P value**	**Estimate OR**	**95%CI**
Sex	0.02	0.77	0.61-0.96	0.47	0.90	0.69-1.19
Age	<0.0001	1.07	1.06-1.08	<0.0001	1.07	1.06-1.08
BMI, Kg/m^2	0.006	1.04	1.01-1.08	0.18	1.02	0.98-1.06
Smoking history [a]	0.37	1.12	0.87-1.43	NA	NA	NA
Drinking history [b]	0.045	1.30	1.01-1.69	0.15	1.26	0.92-1.71
Family history of cancer	<0.0001	2.07	1.51-2.86	<0.0001	2.63	1.81-3.86
History of diabetes	<0.0001	4.36	3.02-6.42	<0.0001	2.30	1.55-3.47

[a] Current and former smoking history was considered as ever. [b] Drinking at least once a month or for more than half a year was considered as ever. $p < 0.05$ indicated significance. BMI was calculated by dividing a person's weight by the square of their height. OR = odds ratio. NA = not applicable.

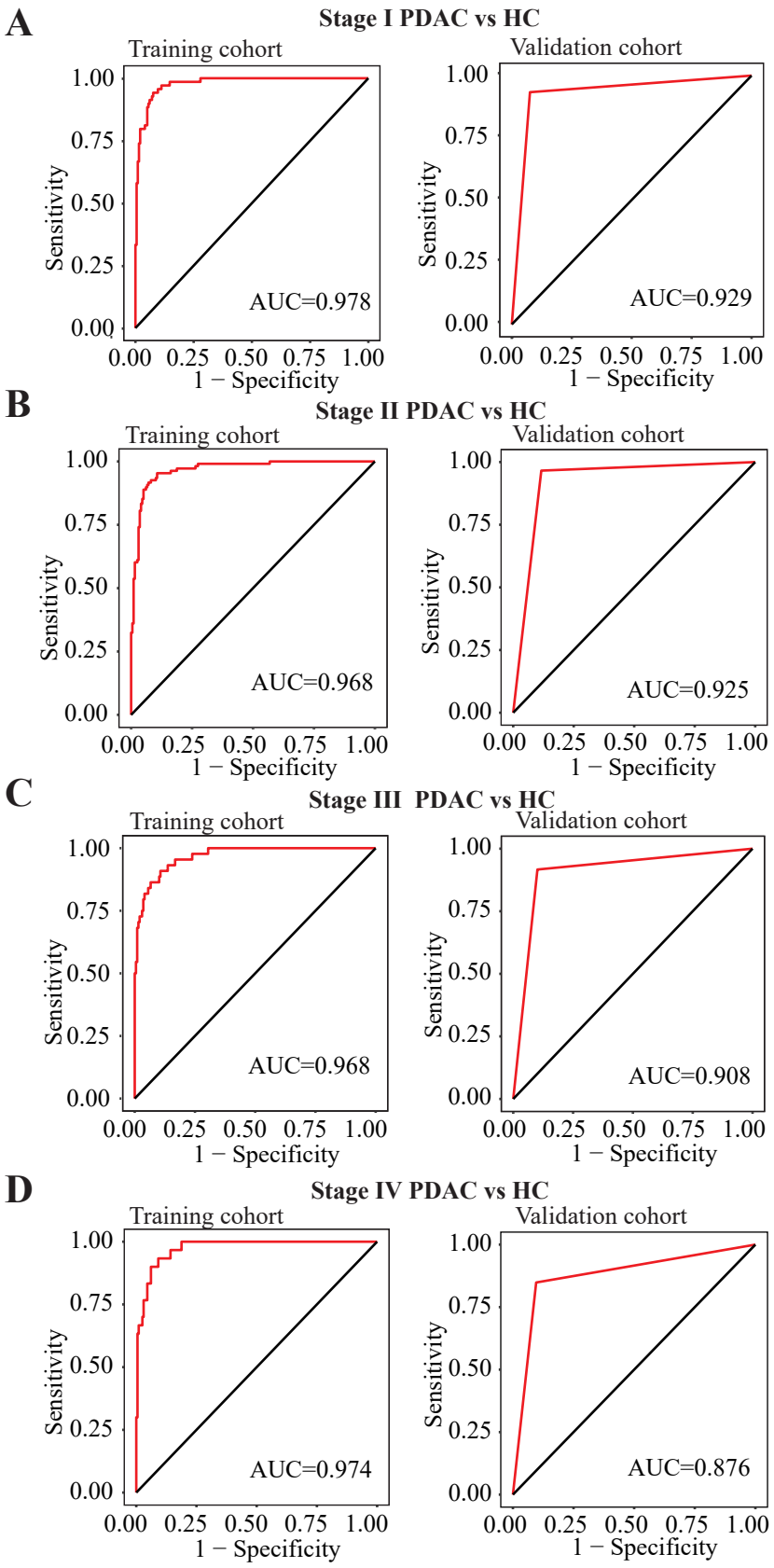
Supplementary Figures



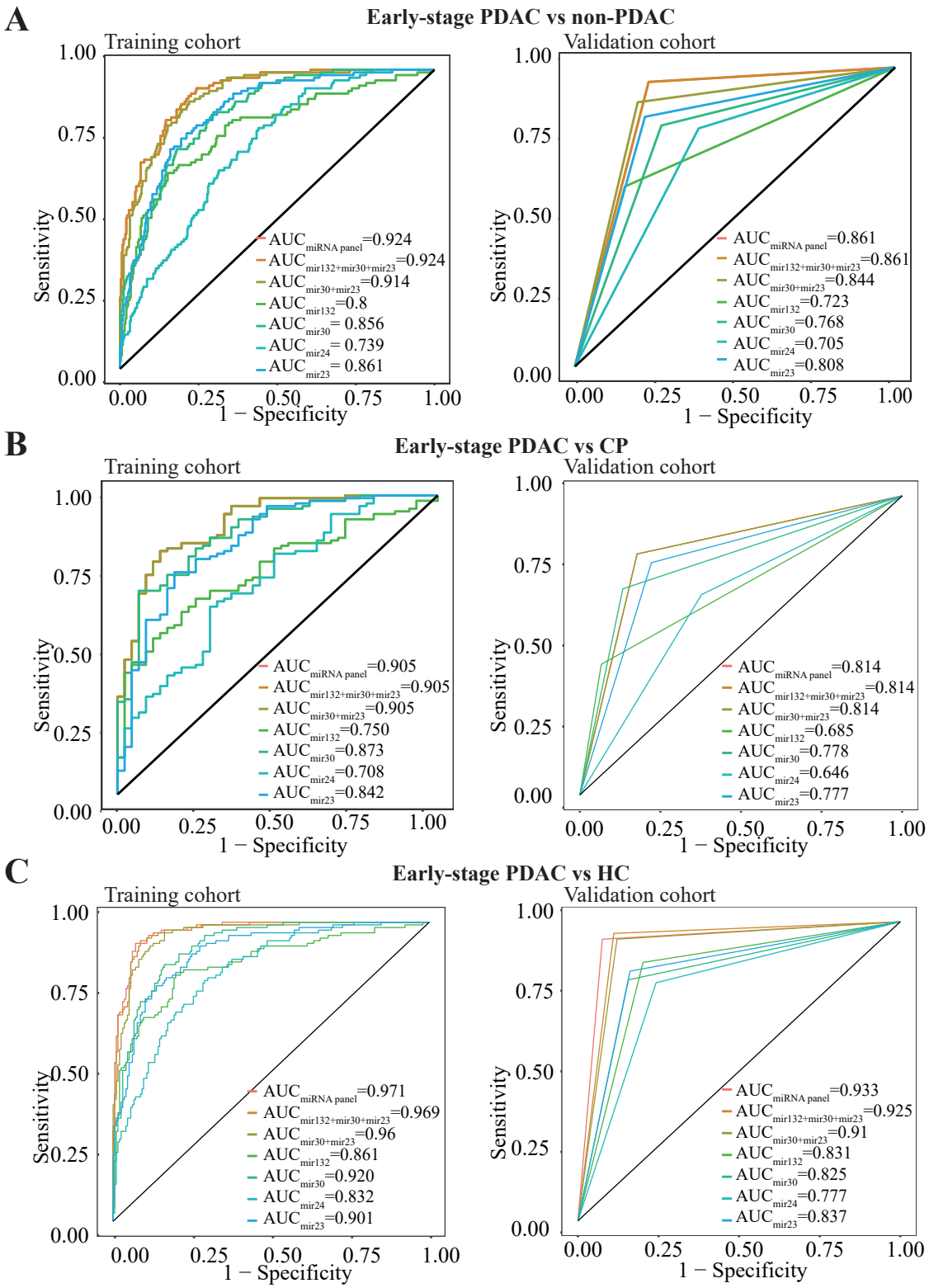
Supplementary Figure 1. Biomarker discovery of miRNAs in FFPE tissues. (**A**) Heat map of all miRNAs identified by microarray expression profiling in FFPE tissues (n = 300) from benign, early lesions (PanIN-TNM stage IIA), and advanced stages (>IIA). Each group contained 100 tissue cores, with statistical power ≥95%. $p<0.05$. (**B**) Quantitative polymerase chain reaction analysis of differentially expressed miRNAs in benign, early lesions and advanced stage tissues. Each group was duplicated with nine samples. Delta delta Ct value was the relative delta Ct value of each group normalized to the mean delta Ct value of benign gruop. The column and bar represents mean with ±SD. The significance was analyzed with one-way ANOVA: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.



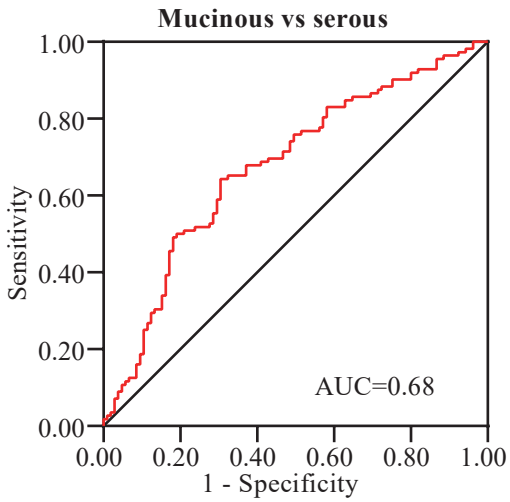
Supplementary Figure 2. Performance of the miRNA panel in the detection of PCa. ROC curves of the miRNA panel in distinguishing patients with PDAC from non-PDAC subjects (HCs and individuals with CP and PCN) (**A**), patients with CP (**B**), and HCs (**C**) in the training and validation cohorts.



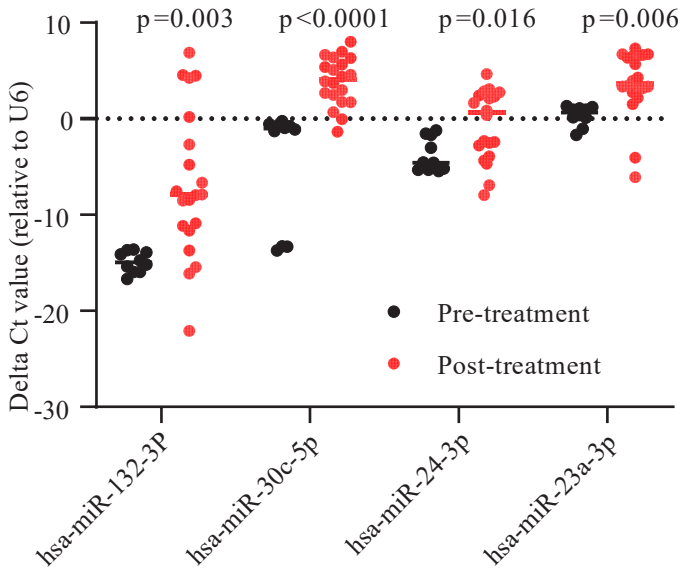
Supplementary Figure 3. Performance of the miRNA panel in the detection of different stages of PCa. ROC curves of the miRNA panel in distinguishing patients with PDAC at TNM stage I (**A**), II (**B**), III (**C**), and IV (**D**) from HCs in the training and validation cohorts.



Supplementary Figure 4. Performance of the four miRNAs individually or in combination in the detection of early-stage PCa. ROC curves of the four miRNAs individually or in combination in distinguishing patients with early-stage PDAC (TNM stage≤ IIA) from non-PDAC (healthy controls and individuals with CP and other pancreatic diseases) (**A**), CP (**B**), and HC (**C**) in the training and validation cohorts.



Supplementary Figure 5. Performance of the miRNA panel in discrimination of pancreatic mucinous tumors from serous tumors. ROC curve of the four-miRNA panel in distinguishing patients with pancreatic mucinous tumors from serous tumors.



Supplementary Figure 6. Expression levels of four individual miRNAs in patients with PCa before or after receiving treatment. 10 pre-treatment patients and 20 post- treatment patients with PCa were detected with dual-channel RT-PCR. MiRNA expression levels were represented as the delta Ct value, which was the relative Ct value of each miRNA normalized to U6. The significance was analyzed with unpaired t-test: ****, p<0.0001.