Supplemental Table 1. Characteristics of PDAC cell lines used for NanoVelcro Chip calibration, efficiency assessment, and feasibility of single cell mutational analysis.

PDAC Cell Line	Source	Differentiation	EpCAM Expression	*KRAS* Mutation	Zygosity
CFPAC-1	Metastasis	Well	High	G12V	Heterozygous
BxPC-3	Primary	Moderate	Moderate	WT*	WT*
PANC-1	Primary	Poor	Low	G12D	Heterozygous
AsPC-1	Metastasis	Poor	Moderate	G12D	Homozygous

*WT = Wild-type

Supplemental Table 2. KRAS amplification and mutation status frequencies of CTCs and WBCs analyzed. The zygosity found from the single cell KRAS sequencing trace files is listed.

Patient	Cell Type	*KRAS* Amplified Cells (#)	*KRAS* Mutated Cells (#)	*KRAS* Mutation CTC	Homozygous	Heterozygous
A	CTC	17	10	G12D	8	2
	WBC	7	0	WT		
B	CTC	6	2	G12V	2	0
	WBC	4	0	WT		
C	CTC	3	3	G12D	3	0
	WBC	2	0	WT		
D	CTC	14	4	G12D	2	2
	WBC	6	0	WT		
E	CTC	4	2	G12D	2	0
	WBC	2	0	WT		

Patient (#)	Cell Type	Total (#)	Total (#)	*KRAS* Mutation		
All	CTC*	44	21	G12V, G12D	17	4
All	WBC*	21	0	WT*		

*CTC = Circulating Tumor Cell (Nuc+/CK+ and/or CEA+/CD45-), WBC = White Blood Cell (Nuc +/CK-/CEA-/CD45+), Nuc = Nucleus, WT = Wild Type

Supplemental Table 3. Comprehensive database for enrolled patients. The table shows all patients and information used to calculate statistics and diagnostic parameters. Patients are listed in chronological order based on the date they presented to the UCLA Center for Pancreatic Diseases.

Patient	Diagnosis[d]	EUS/FNA[d]	Pathology (Surg/Core Bx)*	Surgery	AJCC Stage	Tumor Size (cm)	Tumor Grade	Node Status	CTC Count per 4mL	CK+/CEA- CTCs	CEA+/CK- CTCs	CK+/CEA+ CTCs	CA 19-9 (U / mL)
1	Non-PDAC		Serous Cyst	Whipple		4			0	No	No	No	
2	Non-PDAC	Non-Diagnostic	MCN[d]	Distal Panc		3.2			0	No	No	No	
3	PDAC	Non-Diagnostic	PDAC	Whipple	2	3.6	1	Negative	6	Yes	No	Yes	1846
4	PDAC	PDAC			4	3.1			10	Yes	No	Yes	3390
5	PDAC	PDAC	PDAC	ExLapMets	4	2.8			48	Yes	No	No	4207
6	PDAC	PDAC		ExLapUnrsx	3	3.4			1	No	Yes	No	1263
7	PDAC		PDAC	Whipple	2	1.5	2	Positive	2	Yes	No	Yes	2504
8	PDAC	Non-Diagnostic	PDAC	Whipple	2	1.8	2	Positive	2	Yes	No	Yes	28
9	PDAC	PDAC			3	3			0	No	No	No	1641
10	PDAC	PDAC	PDAC	Distal Panc	2	4.6	2	Positive	0	No	No	No	327
11	PDAC	PDAC			4	1.2	1		9	Yes	No	No	160
12	Non-PDAC	Complex Cyst				5			1	Yes	No	No	
13	PDAC	PDAC			3	3.7			1	Yes	No	No	1096
14	PDAC	PDAC	PDAC	Whipple	2	4.4	3	Positive	0	No	No	No	27
15	Non-PDAC	Atypical	MD IPMN[b]	Whipple		2.7			0	No	No	No	
16	PDAC	PDAC			4	3.8	2		16	Yes	No	No	3817
17	PDAC	PDAC			4	2.5	3		10	Yes	No	Yes	621
18	PDAC	PDAC			4	2.7			5	Yes	No	Yes	3317
19	PDAC	PDAC			4	1.5			5	Yes	Yes	Yes	180
20	PDAC	PDAC			3	5.8			2	No	No	Yes	
21	PDAC	PDAC	PDAC	ExLapMets	4		2		10	No	Yes	No	
22	Non-PDAC	Inflammation/Atypical	Chronic Pancreatitis						0	No	No	No	
23	PDAC	PDAC	PDAC	Whipple	2	2	1	Positive	2	No	No	Yes	7
24	Non-PDAC	No Lesion							0	No	No	No	
25	Non-PDAC	SB IPMN*[a]				3.5			0	No	No	No	
26	Non-PDAC	SB IPMN*[a]				3.2			0	No	No	No	
27	PDAC	PDAC	PDAC		4	6.6	3		2	No	No	Yes	65500
28	PDAC	PDAC	PDAC	Whipple	2	10.5	2	Positive	2	Yes	No	No	980
29	PDAC	PDAC	PDAC	Whipple	2	4.5	2	Positive	1	No	Yes	No	94
30	PDAC	PDAC			4				0	No	No	No	25684
31	PDAC	PDAC	PDAC		3	4.2			1	No	Yes	No	1424
32	PDAC	PDAC	PDAC	ExLapMets	4	3.9	3		1	Yes	No	No	16
33	Non-PDAC		Pseudocyst	Distal Panc		3.6			0	No	No	No	
34	Non-PDAC	MD IPMN*[b]				1.2			0	No	No	No	
35	PDAC	PDAC			3	3.5			1	No	Yes	No	3
36	Non-PDAC	MD IPMN*[b]				2.3			0	No	No	No	
37	Non-PDAC		Serous Cyst	Whipple		1.9			0	No	No	No	
38	Non-PDAC	PNET[c]	PNET[c]	Distal Panc		0.5	1	Negative	0	No	No	No	
39	PDAC	PDAC			4	5.4			1	Yes	No	No	119042
40	PDAC	PDAC	PDAC	ExLapMets	4	3.2	3		3	No	No	Yes	679
41	PDAC	PDAC	PDAC		3	2.3	3		2	No	No	Yes	159
42	Non-PDAC	Serous Cyst*				1.8			0	No	No	No	
43	Non-PDAC	PNET[c]	PNET[c]	Distal Panc		1.4	1	Negative	0	No	No	No	
44	PDAC	PDAC			2	3.5	1		1	Yes	No	No	
45	PDAC	PDAC	PDAC	Distal Panc	1	6.6	1	Negative	0	No	No	No	361
46	Non-PDAC	MD IPMN*[b]				2			0	No	No	No	
47	PDAC	PDAC			4	5.3	3		3	Yes	No	Yes	765
48	Non-PDAC	Atypical	MD IPMN[b]	Whipple		4			0	No	No	No	
49	Non-PDAC	No Lesion							0	No	No	No	
50	PDAC	PDAC			3	2			0	No	No	No	
51	PDAC	PDAC	PDAC	Whipple	2	3.9	2	Positive	1	Yes	No	No	2
52	Non-PDAC	SB IPMN*[a]				2.4			0	No	No	No	
53	PDAC	Non-Diagnostic	PDAC	Whipple	2	1.8	2	Negative	0	No	No	No	
54	PDAC		PDAC	Whipple	2	2.1	2	Negative	2	No	No	Yes	24
55	PDAC	PDAC			2	2.9			0	No	No	No	2870
56	PDAC		PDAC	ExLapUnrsx	3				1	Yes	No	No	64
57	PDAC	PDAC	PDAC	Whipple	2	3.8			2	No	No	Yes	1244
58	PDAC	PDAC	PDAC	ExLapMets	4	4.2	2		13	Yes	No	Yes	1845
59	Non-PDAC	MD IPMN*[b]	MD IPMN[b]	Distal Panc		2.6			0	No	No	No	
60	Non-PDAC	SB IPMN*[a]				2.3			0	No	No	No	
61	PDAC	PDAC	PDAC	ExLapUnrsx	3	2.2		Positive	3	Yes	No	Yes	3
62	PDAC	PDAC	PDAC	Whipple	2	2.7	2	Positive	1	No	No	Yes	306
63	Non-PDAC	MD IPMN*[b]	MD IPMN[b]	Whipple		1.8			0	No	No	No	
64	PDAC	Non-Diagnostic	PDAC	ExLapMets	4				14	Yes	No	No	
65	PDAC	PDAC			4				4	Yes	No	No	
66	PDAC	PDAC	PDAC	Distal Panc	1	1.8	1	Negative	0	No	No	No	12
67	PDAC	PDAC			3	4.6			0	No	No	No	
68	PDAC	PDAC	PDAC	Whipple	2	1.9	3	Positive	0	No	No	No	103
69	Non-PDAC	Non-Diagnostic	MD IPMN[b]	Whipple		0.7			0	No	No	No	
70	PDAC	PDAC	PDAC	Whipple	2	5	3	Positive	0	No	No	No	222
71	PDAC	PDAC	PDAC	Whipple	2	2.3	2	Positive	0	No	No	No	35
72	PDAC	PDAC			4				5	Yes	No	No	
73	PDAC	PDAC			4	4			47	Yes	No	No	43
74	PDAC		PDAC		4	5.4			4	Yes	No	Yes	111074
75	PDAC	PDAC	PDAC	ExLapMets	4	2.3			3	Yes	No	No	36
76	PDAC	Non-Diagnostic	PDAC	Whipple	2	3.5	2	Positive	1	Yes	No	No	1008
77	Non-PDAC	Non-Diagnostic	MD IPMN[b]	Whipple	0				0	No	No	No	
78	Non-PDAC	MD IPMN*[b]	MD IPMN[b]	Whipple	0				0	No	No	Yes	
79	PDAC	PDAC	PDAC	Whipple	2	2.1	2	Negative	0	No	No	No	
80	PDAC	Non-Diagnostic	PDAC	ExLapMets	4				4	Yes	No	No	668
81	PDAC	PDAC	PDAC		4	2.9			3	Yes	No	No	42
82	PDAC	PDAC	PDAC	Distal Panc	2	5	2	Positive	1	Yes	No	No	3
83	PDAC	PDAC	PDAC	Whipple	2	3.7	2	Positive	0	No	No	No	3
84	PDAC	PDAC	PDAC	ExLapUnrsx	3	1.9			6	No	No	Yes	1110
85	PDAC	PDAC			2	1.9			2	Yes	No	No	
86	PDAC	PDAC	PDAC	Whipple	2	3.6	2	Positive	0	No	No	No	1
87	Non-PDAC		MD IPMN[b]	Whipple	0				0	No	No	No	
88	PDAC	PDAC	PDAC		4	6.9	3		3	Yes	No	Yes	24
89	PDAC	PDAC	PDAC	Whipple	2	2.6	2	Positive	1	Yes	No	No	168
90	PDAC	PDAC	PDAC	Distal Panc	2	5.5	3	Positive	1	Yes	No	No	
91	PDAC	PDAC			4				7	Yes	No	No	121
92	PDAC		PDAC	Whipple	1	2.8	2	Negative	0	No	No	No	
93	Non-PDAC	Serous Cyst*	Serous Cyst	Whipple	0				0	No	No	No	
94	Non-PDAC		MCN[d]	Distal Panc	0				0	No	No	No	
95	PDAC	PDAC			4	4			6	No	No	Yes	386
96	PDAC	PDAC	PDAC	Whipple	2	4.1	2	Positive	3	No	No	Yes	415
97	PDAC	PDAC			2	1.7			0	No	No	No	29
98	PDAC	PDAC			4				6	No	No	Yes	
99	PDAC	PDAC		ExLapUnrsx	3	4			9	No	No	Yes	2261
100	PDAC	PDAC	PDAC	ExLapUnrsx	3				4	Yes	No	Yes	137

*Indicates probable diagnosis based on image appearance, EUS characterization, lack of malignant cells on FNA, and cyst fluid analysis.
a: SB IPMN = Side Branch Intraductal Papillary Mucinous Neoplasm, b: MD IPMN = Main Duct Intraductal Papillary Mucinous Neoplasm, c: PNET = Pancreatic Neuroendocrine Tumor, d: MCN = Mucinous Cystic Neoplasm, e: PDAC = Pancreatic Ductal Adenocarcinoma

Supplemental Table 4. Diagnostic parameters for CTCs and CA 19-9 at various cut-off values.

Diagnostic Parameters for Metastatic Disease

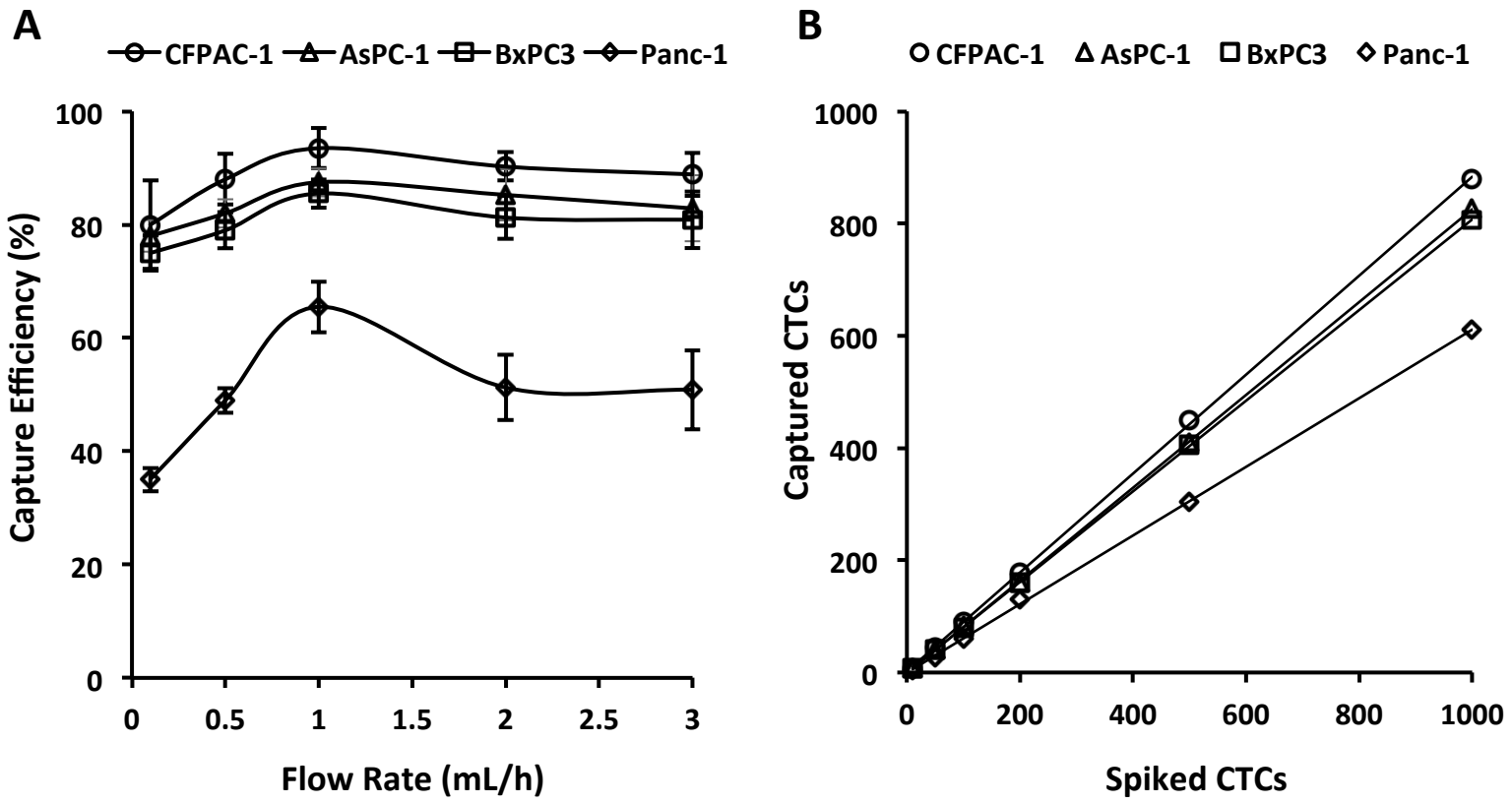
CTC Cut-Off	Sensitivity	Specificity	PPV	NPV	Youden's Index
≥ 1 CTC	0.963	0.378	0.481	0.944	0.341
≥ 2 CTC	0.889	0.667	0.615	0.909	0.556
≥ 3 CTC	***0.852***	***0.867***	***0.793***	***0.907***	***0.719***
≥ 4 CTC	0.667	0.911	0.818	0.820	0.578
≥ 10 CTC	0.296	1.000	1.000	0.703	0.296

Diagnostic Parameters for Metastatic Disease

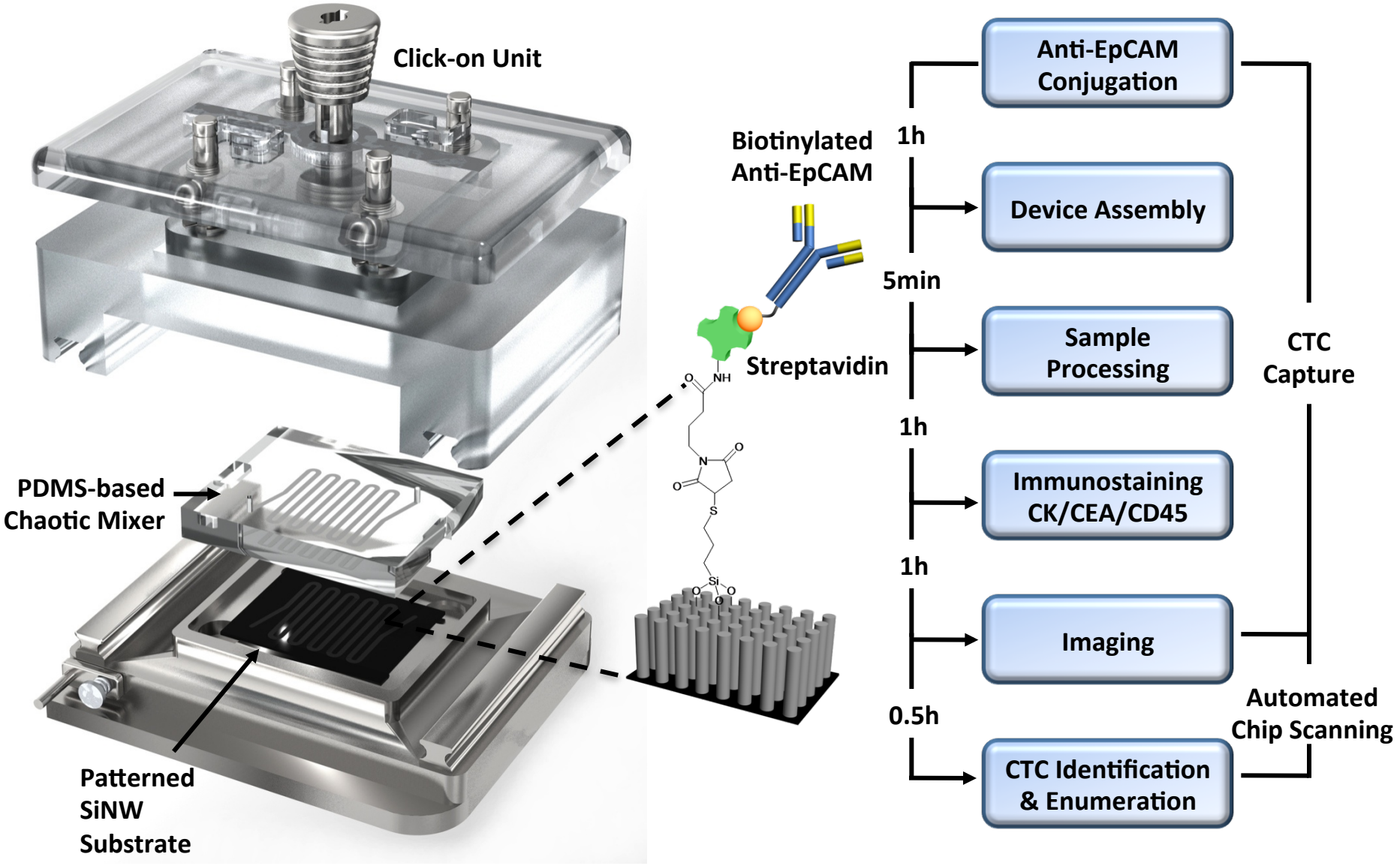
CA19-9 Cut-Off	Sensitivity	Specificity	PPV	NPV	Youden's Index
≥ 100	0.783	0.417	0.462	0.750	0.199
≥ 500	0.609	0.667	0.538	0.727	0.275
≥ 1000	0.391	0.694	0.450	0.641	0.086
≥ 2500	0.348	0.944	0.800	0.694	0.292
≥ 3000	***0.348***	***1.000***	***1.000***	***0.706***	***0.348***

Diagnostic Parameters for PDAC

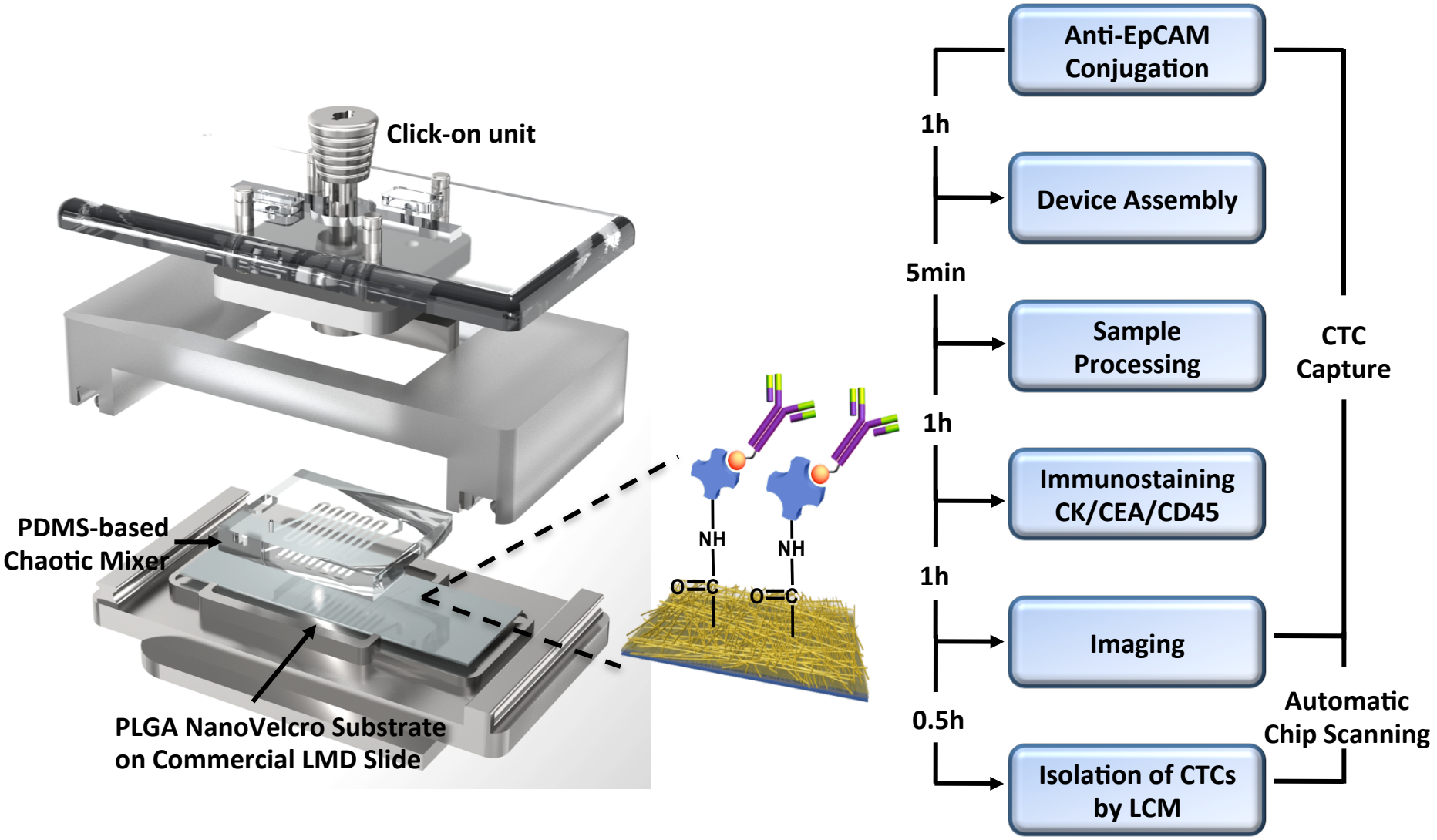
CTC Cut-Off	Sensitivity	Specificity	PPV	NPV	Youden's Index
≥ 1 CTC	***0.750***	***0.964***	***0.982***	***0.600***	***0.714***
≥ 2 CTC	0.542	1.000	1.000	0.450	0.542
≥ 3 CTC	0.403	1.000	1.000	0.394	0.403
≥ 4 CTC	0.306	1.000	1.000	0.231	0.306
≥ 10 CTC	0.111	1.000	1.000	0.207	0.111



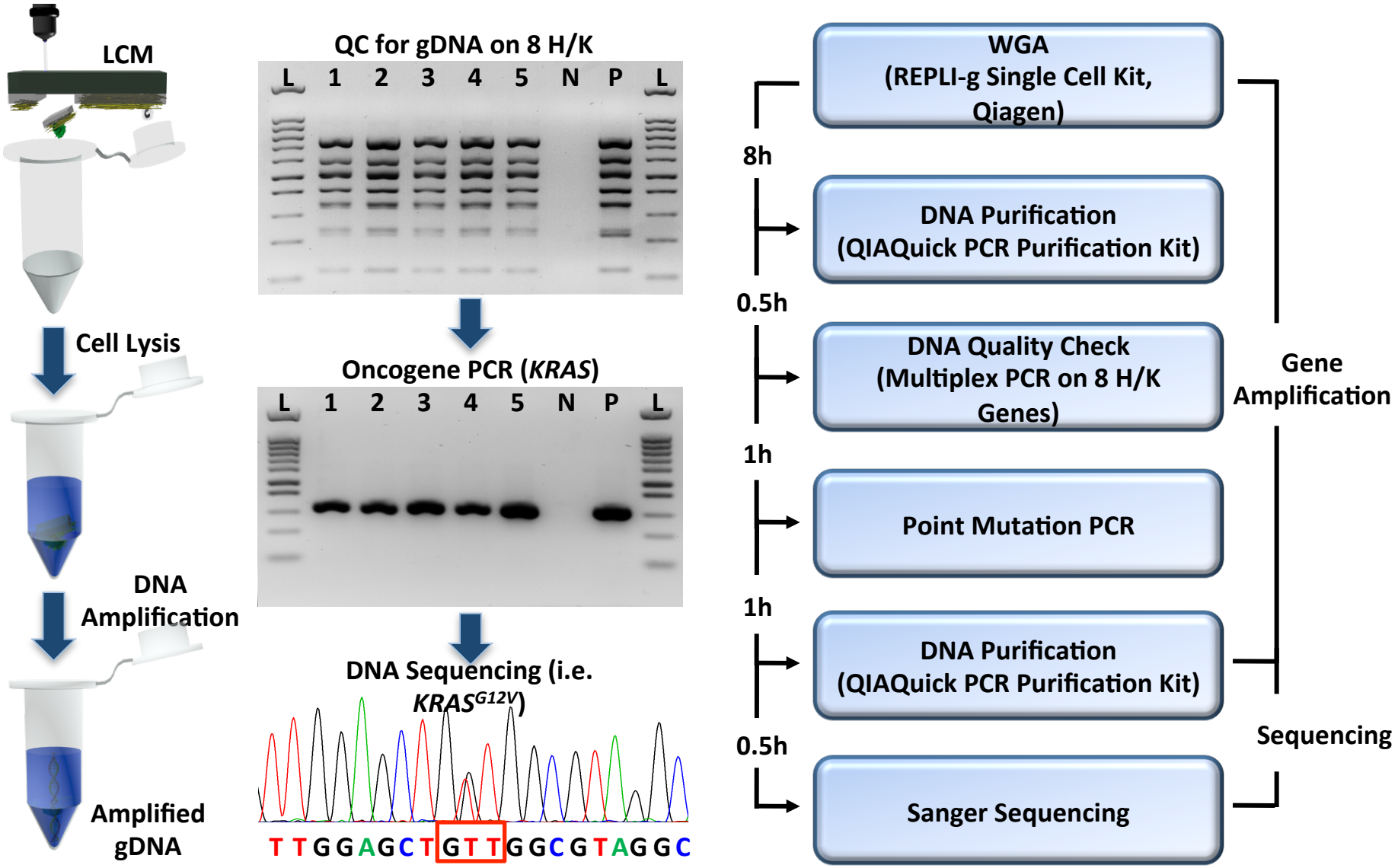
Supplemental Figure 1. Calibration curves and capture efficiency data for PDAC cell lines. **(A)** Diagram of capture efficiency for CFPAC-1, AsPC-1, BxPC-3, and PANC-1 as a function of NanoVelcro device flow rates. **(B)** Capture efficiency of CFPAC-1, AsPC-1, BxPC-3, and PANC-1 as a function of the number of captured cells versus the number spiked into the artificial sample.



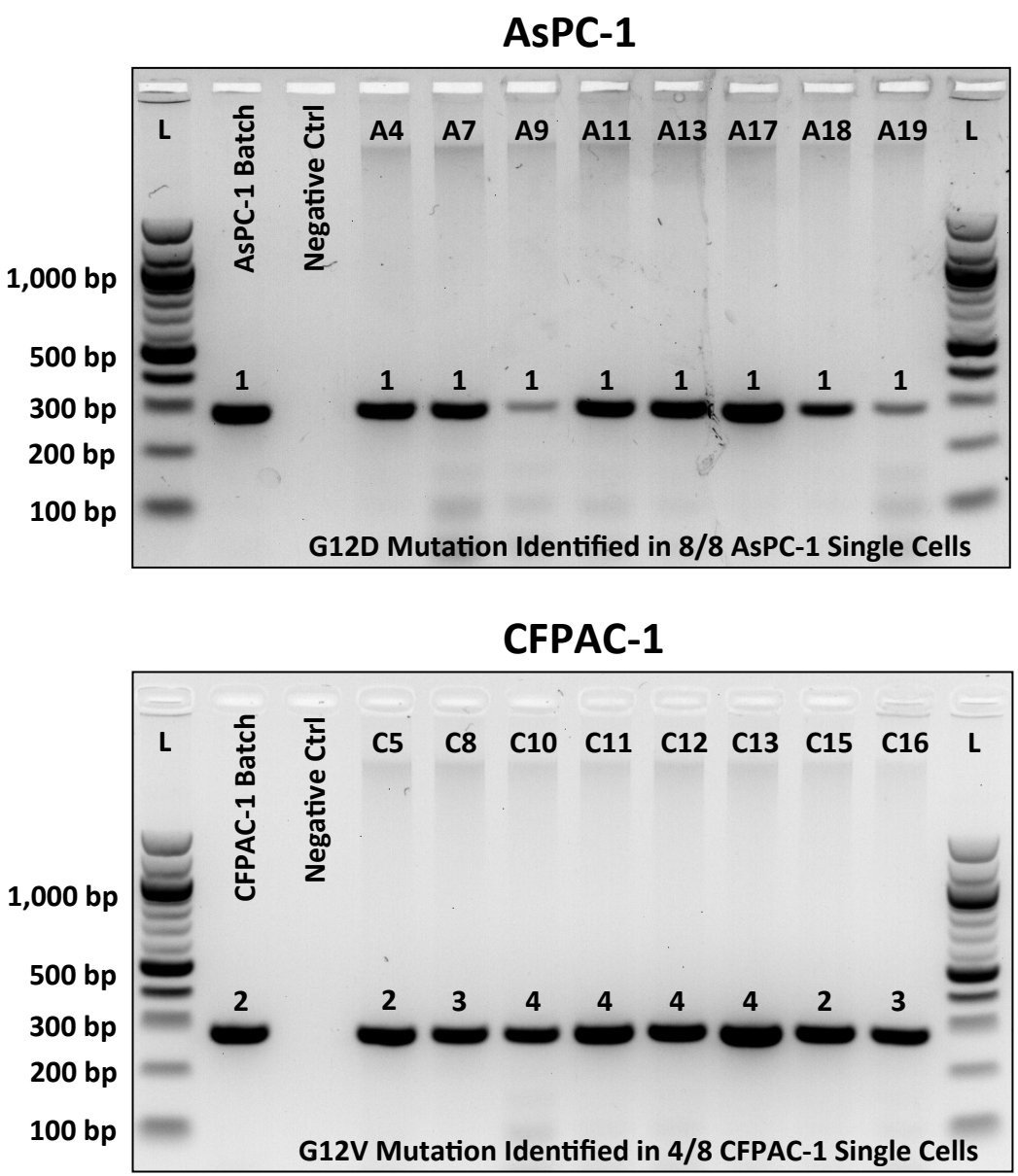
Supplemental Figure 2. General workflow diagram for CTC capture, identification, and enumeration on SiNS NanoVelcro Chips. The schematic shows the individual components of SiNS NanoVelcro Chips, chip holding device, and elements/duration of the workflow for processing a typical blood sample.

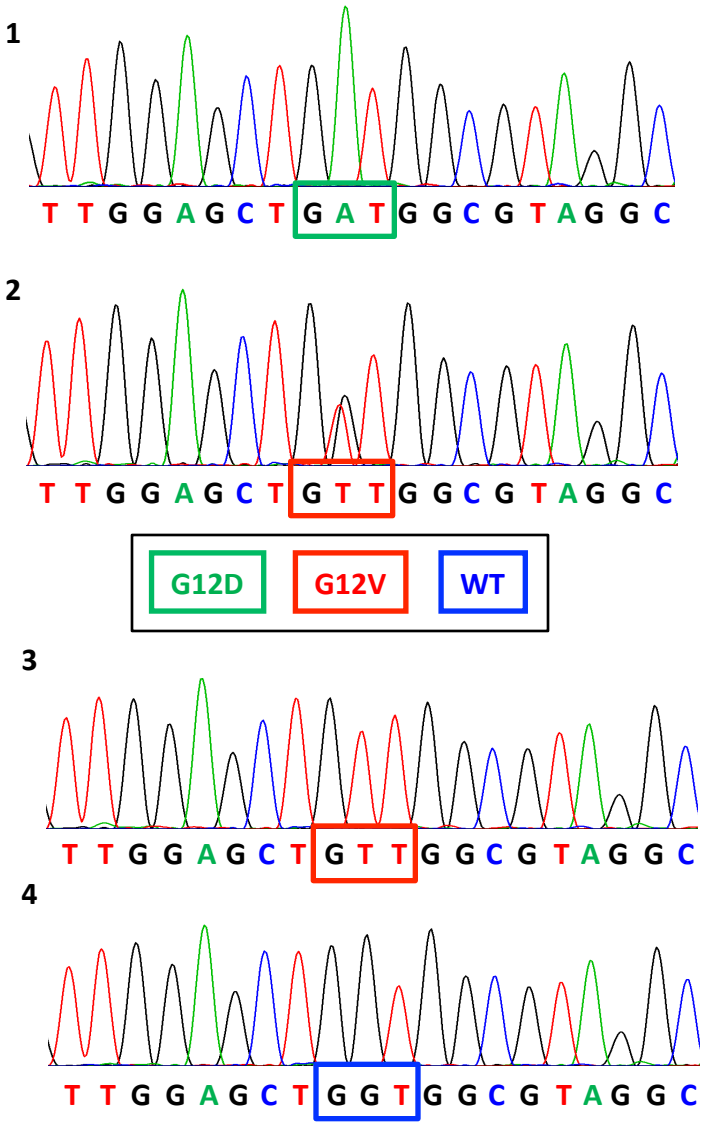


Supplemental Figure 3. General workflow diagram for CTC capture, identification, and LCM of single CTCs on NanoVelcro/LCM Chips. Individual components of the NanoVelcro/LCM chips and holding device are shown in the schematic at the right.

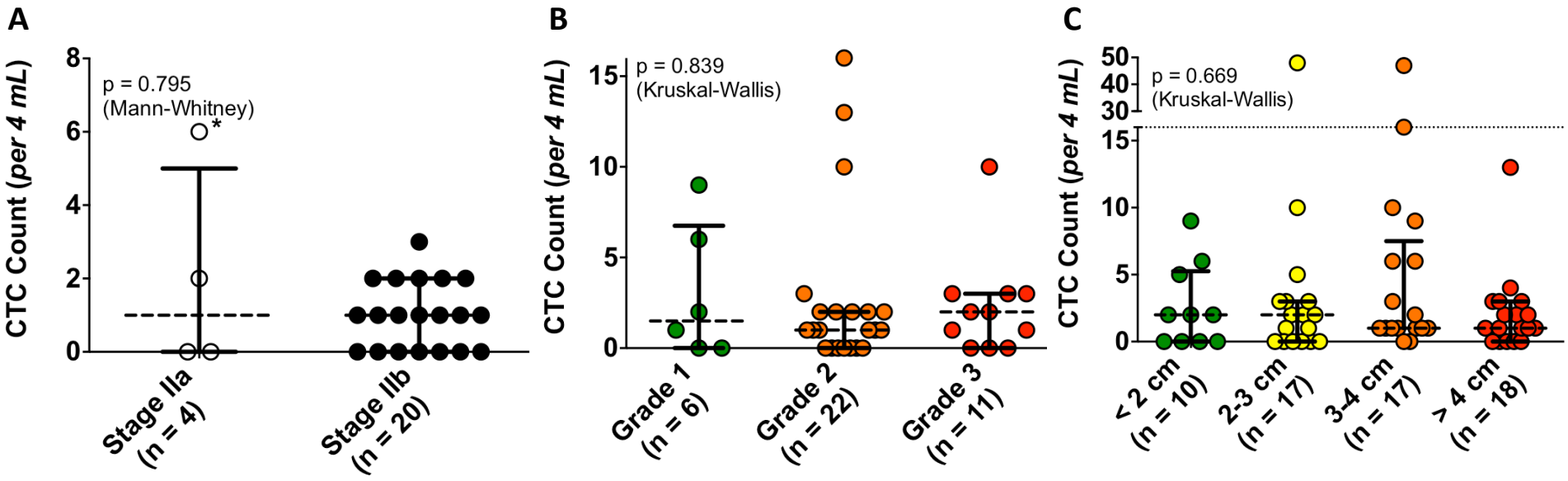


Supplemental Figure 4. General workflow diagram for the process of single-CTC whole genome amplification, DNA quality assessment, and targeted mutational analysis of *KRAS* via PCR and Sanger Sequencing. H/K Genes – Housekeeping Genes





Supplemental Figure 5. Targeted mutational analysis of *KRAS* codon 12 via PCR and Sanger Sequencing in PDAC cell lines. *KRAS* PCR of batch cellular DNA and WGA of single cells are shown in the agarose gels at the left for AsPC-1 (G12D Homozygote) and CFPAC-1 (G12V Heterozygote). The expected PCR product shows a band at ~300 bp. The accompanying chromatograms shown at the right are representations of the different Sanger Sequencing results obtained. *JKRAS* mutation subtype is indicated by the colored boxes. The numeric labels over the bands in the agarose gels indicate the corresponding chromatogram/sequencing result shown on the right.



Supplemental Figure 6. Additional statistical analysis. **(A)** Comparison of CTC counts between node-negative (Stage IIa) and node-positive (Stage IIb) patients reveals no significance (p = 0.795). **(B)** Comparison of CTC counts between patients with different tumor grades reveals no significance (p = 0.839). **(C)** Comparison of CTC counts and tumor size reveals no significance (p = 0.669).