Supporting Information for

Microfluidic Isolation of Circulating Tumor Cells and Cancer Stem-like Cells from Patients with Pancreatic Ductal Adenocarcinoma

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Table S1. Patient demographics and clinicopathological characteristics.

Demographic	All Patients (N=24)	
	No.	%
Age (years)		
Mean	64.8	
Standard deviation	10.4	
Min – Max	42 – 82	
Gender		
Male	17	70.8
Female	7	29.2
Race		
White	23	95.8
Asian	1	4.2
Ethnicity		
Non-Hispanic	23	95.8
Hispanic	1	4.2
Tumor Location in Pancreas		
Head	9	37.5
Body	6	25.0
Tail	4	16.7
Body/Head	2	8.3
Body/Tail	1	4.2
Unspecified	2	8.3
Baseline Serum CA19-9 Levels		
Mean	2894.1	
Standard deviation	4220.8	
Sample size	78	
Enumeration	CTCs (EpCAM)	CSCs (CD133)
Sample size	77	72
Mean (per mL)	3.87	2.02
Standard deviation (per mL)	2.43	1.73
Min – Max (per mL)	0 – 9.25	0 – 9.25

Table S2. Fluorescent filter cube setup (from Chroma Technology Corporation).

Filter cubes	DAPI	FITC	CY3	CY5
Excitation (EX)	AT350/50x	HQ480/40x	ET545/25x	ET620/60x
Beam splitter (BS)	T400lp	T510lpxrxt	T565lpxr	T660lpxr
Emission (EM)	ET460/50m	ET525/30m	ET605/70m	ET700/75m
Set catalog #	49000	49011*	49004	49006

*Only the original excitation filter was used from this product catalog, the beam splitter and emission filter were replaced to create a custom FITC filter cube.

Table S3. Number of EpCAM$^+$CK$^+$CD45$^-$DAPI$^+$ cells and CD133$^+$CK$^+$CD45$^-$DAPI$^+$ cells detected in healthy donor blood. The healthy control counts are represented in Figure 2.

Capture Antibody Used	Healthy Donor Blood Sample	CK$^+$CD45$^-$DAPI$^+$ cells/mL
Anti-EpCAM	1	0
	2	0
	3	0
	4	0
	5	0
	6	0
	7	0
	8	0
	9	0
	10	0
	11	0.25
	12	0
	13	0
	14	0
	15	0.25
	16	0
	17	0
	18	0.75
	19	0
Anti-CD133	1	0
	2	0
	3	0
	4	0
	5	0

Table S4. Quantification of CTCs in metastatic pancreatic cancer patients. The patient CTC (N=61) and CSC (N=56) data represented in Figure 2 is shown in the table below.

Patient ID	Treatment Cycle #	EpCAM$^+$CK$^+$CD45$^-$ DAPI$^+$ cells/ mL	CD133$^+$CK$^+$CD45$^-$ DAPI$^+$ cells/mL
P-07	41	4	3
P-07	62	1.5**	0**
P-07	65	2.75	0.5
P-07	78	2.25	0.75
P-07	79	2	0
P-15	32	1	2.5
P-15	43	3.75	2.75
P-15	44	4	2.25
P-15	48	3	2
P-15	63	1.5	1
P-15	65	1.5	0.5
P-15	73	1	0
P-19	7	6	N/A
P-19	8	3	N/A
P-19	10	3.25	0.5
P-19	12	5.25	0.75
P-19	18	4.5	N/A
P-19	20	2.55	N/A
P-19	22	3.125	N/A
P-19	24	2.1875	N/A
P-19	26	N/A	3
P-24	10	8	2.5
P-24	12	6.5	5
P-29	1	9.25	1.5
P-30	1	5.75	9.25
P-30	3	3	0
P-30	13	4	2
P-30	15	5.5	4.25
P-30	16	4.75	4.5
P-32	10	3.25	1.5
P-32	12	2.5	1.25
P-32	15	8.25	3
P-32	22	5	2.75
P-33	2	6	4.5
P-34	1	7	3.75
P-34	2	3	3.5
P-34	3	3.25	2.5
P-34	5	2.5	4
P-35	1	8.25	3.5
P-35	2	3.5	3.25

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P-35	5	5	3
P-35	6	6.5	3.75
P-36	1	5.5	0.5
P-36	2	0.75	0.75
P-36	5	2	0
P-36	11	1.25	0
P-36	14	4.25	1
P-37	1	7.5	2.25
P-38	1	4.5	1.25
P-38	2	3	1
P-38	3	3.25	1.75
P-38	4	4	3.5
P-40	1	8.25	4.25
P-40	2	9	2.75
P-40	3	6.5	3
P-41	1	6.25	5.5
P-42	1	7.25	1.75
P-42	2	8.5	1.5
P-43	1	7	0.5
P-43	2	4	0
P-44	1	4.75	2
P-44	2	2	1.5

Note: the asterisks (**) marks the enumeration data point which was obtained using the RBC lysis method, due to technical issues encountered during PBMC isolation.

Table S5. CTC/CSC subtype enumeration. The patient CTC and CSC subtype enumeration data (N=16) from 11 of the 24 patients is shown in the table below. All the patients in the subtype analysis (denoted with a 'C' instead of a 'P') were given different identifiers to blind scientists about the source of the blood. Five of the eleven patients in the subtype analysis were also part of the main CTC and CSC quantification study.

	EpCAM Capture		CD133 Capture		EpCAM & CD133 Capture
Patient ID	$CD133^+CK^+$ $CD45^-DAPI^+$ cells/mL	$CD133^-CK^+$ $CD45^-DAPI^+$ cells/mL	$EpCAM^+CK^+$ $CD45^-DAPI^+$ cells/mL	$EpCAM^-CK^+$ $CD45^-DAPI^+$ cells/mL	CK^+CD45^- $DAPI^+$ cells/mL
C-16 (P-32)	0	2	1	3	N/A
C-23	1	3	0	2	N/A
C-24	0	2	1	1	N/A
C-27	0	0	0	1	N/A
C-27	0	0	0	0	1
C-28	0	0	2	0	0
C-29	2	1	0	0	5
C-30 (P-15)	1	2	0	0	0
C-30 (P-15)	0	1	0	0	N/A
C-30 (P-15)	0	1	0	0	N/A

C-31 (P-35)	0	2	0	0	N/A
C-31 (P-35)	0	4	0	4	0
C-32	0	0	0	0	2
C-33 (P-07)	0	1	1	2	4
C-33 (P-07)	0	0	0	1	N/A
C-38 (P-36)	0	7	0	3	N/A
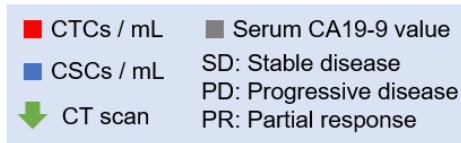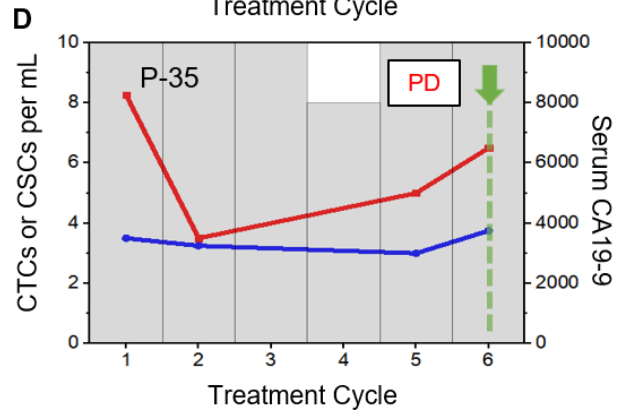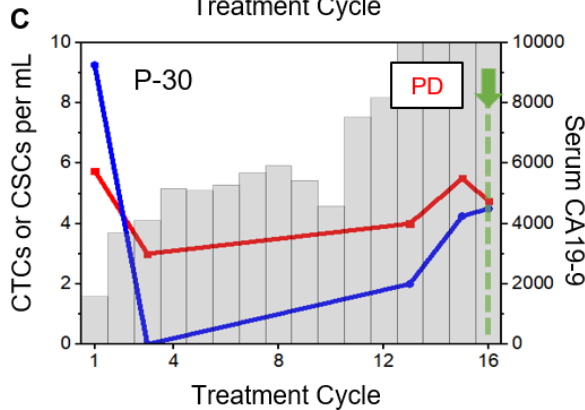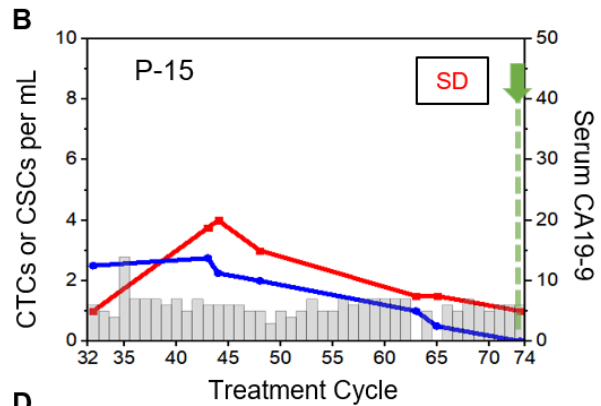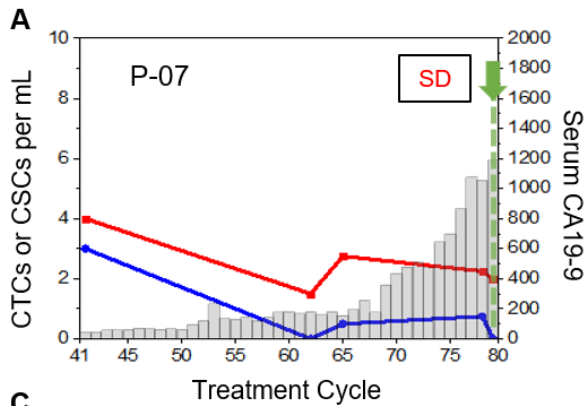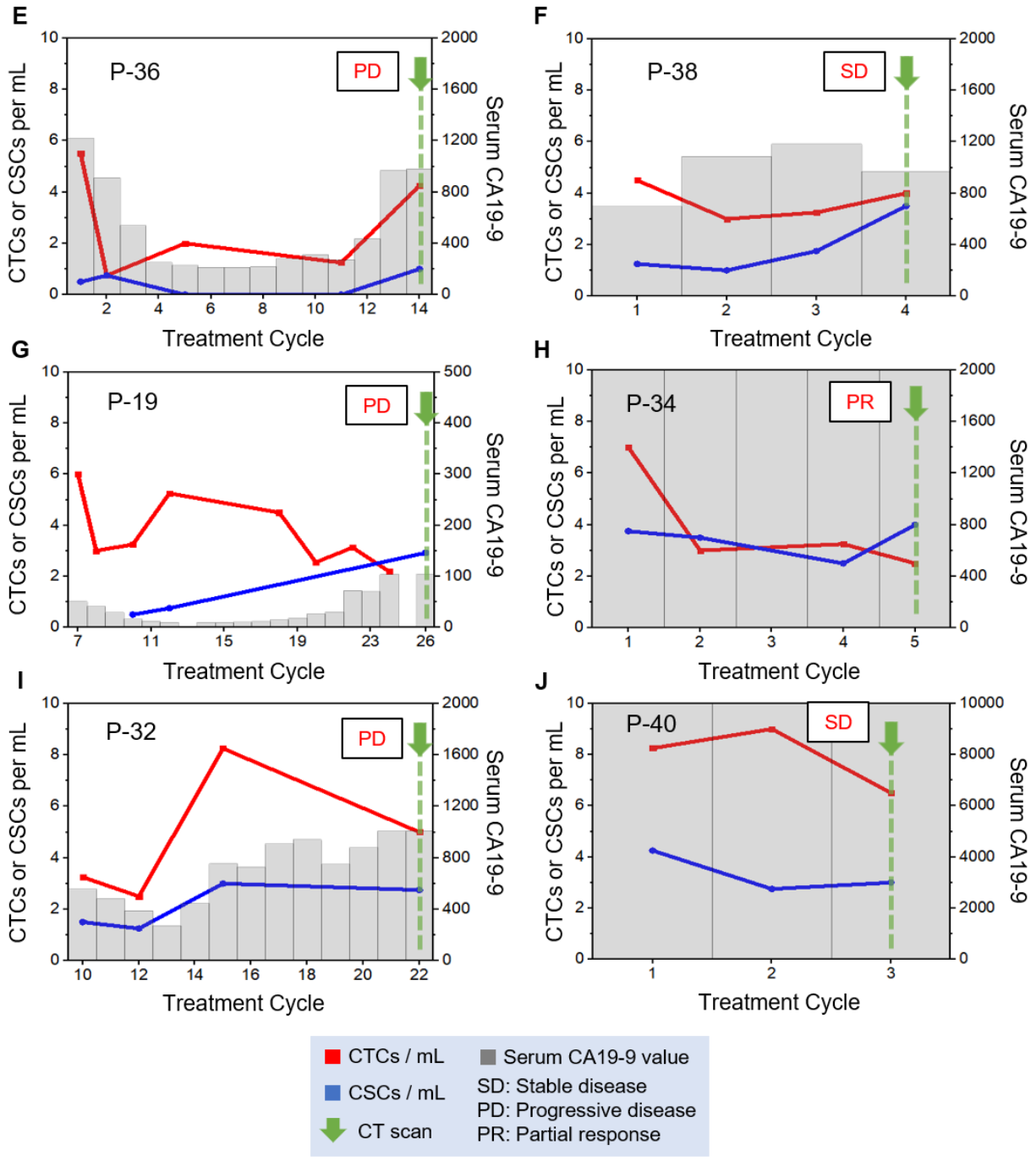
Figure S1. Longitudinal enumeration of CTCs and CSCs during treatment. Graphs A-J show the evolution of CTC and CSC numbers, as well as serum CA19-9 values, during the clinical course and treatment of a set of ten PDAC patients. Green arrows depict CT scans. Each treatment cycle is 2 weeks long.

Table S6. Pearson correlation table.

		CTCs / mL	CSCs / mL	Serum CA19-9	Treatment cycle
CTCs per mL	Correlation coefficient Significance level p-value		0.425 0.0008	0.320 0.0136	-0.503 <0.0001
CSCs per mL	Correlation coefficient Significance level p-value	0.425 0.0008		0.434 0.0006	-0.332 0.0103
Serum CA19-9 levels	Correlation coefficient Significance level p-value	0.320 0.0136	0.434 0.0006		-0.362 0.0048
Treatment cycle	Correlation coefficient Significance level p-value	-0.503 <0.0001	-0.332 0.0103	-0.362 0.0048	