

## **Supplementary figure legends:**

### **Supplementary figure 1:**

a) Comparison of total CTC numbers between tumor location (head vs. tail,  $n=65$ ,  $p=0.99$ ), b) Scatter plot depicting total CTC numbers in metastatic (M1) vs. non-metastatic in treatment-naïve patients ( $n=65$ ,  $p=0.47$ ), c) Comparison of total CTC numbers in different AJCC stages ( $n=65$ ,  $p=0.46$ ), d) Correlation matrix between maximum tumor diameter and total CTC number ( $n=21$ ,  $p=0.33$ ), e) Proportion of pEMT-CTC from total CTC count (Prop-pEMT) comparing metastatic (M1) and non-metastatic patients ( $p=0.0032$ ), f) Proportion of pEMT-CTC from total CTC count across different AJCC stages of PDAC ( $p=0.025$ ), g) Comparison of Prop-pEMT across different location of primary PDAC ( $n=65$ ,  $p=0.04$ ).

### **Supplementary figure 2:**

Comparison of proportion of different CTC subtypes in all draws on treatment ( $n=132$ ) in patients progression under therapy vs. patients without progression (SC:  $p=0.52$ , E:  $p=0.046$ , pEMT:  $p=0.056$ , EMT:  $p=0.008$ ).

### **Supplementary figure 3:**

Analysis of additional Vimentin expressing cells in circulation. a) Bright field and immunofluorescence images of 3 patient samples expressing CD31 or FAP in combination with Vimentin. BF: Bright field, Pan-CK: pan-cytokeratin, CD31: cluster of differentiation 31, Vim: vimentin, EpCAM: epithelial cell adhesion molecule, DAPI: 4',6-Diamidin-2-phenylindol, FAP: fibroblast activation protein, and CD45: Protein tyrosine phosphatase, receptor type C. b) Quantification of cell types presents in a).

### **Supplementary table 1:**

Comparison of different clinico-pathological parameters with CTC subtypes in all draws on therapy ( $n=132$ ).

### **Supplementary table 2:**

Comparison of different clinico-pathological parameters with CTC subtypes in treatment-naïve patients (n=65)

**Supplementary table 3:**

Cox proportional hazard analysis of clinicopathological parameters. A) Overall survival of baseline treatment-naïve patients (n=65), B) Progression-free survival of baseline treatment-naïve patients (n=65) C) Multivariable Cox model for PFS.

**Supplementary table 4:**

Summary of calculated PFS using different proportional cutoffs for E, M, pEMT and SC in BTN patients (n=65).

**Supplementary table 5:**

Summary of calculated OS using different proportional cutoffs for E, M, pEMT and SC in BTN draws (n=65)

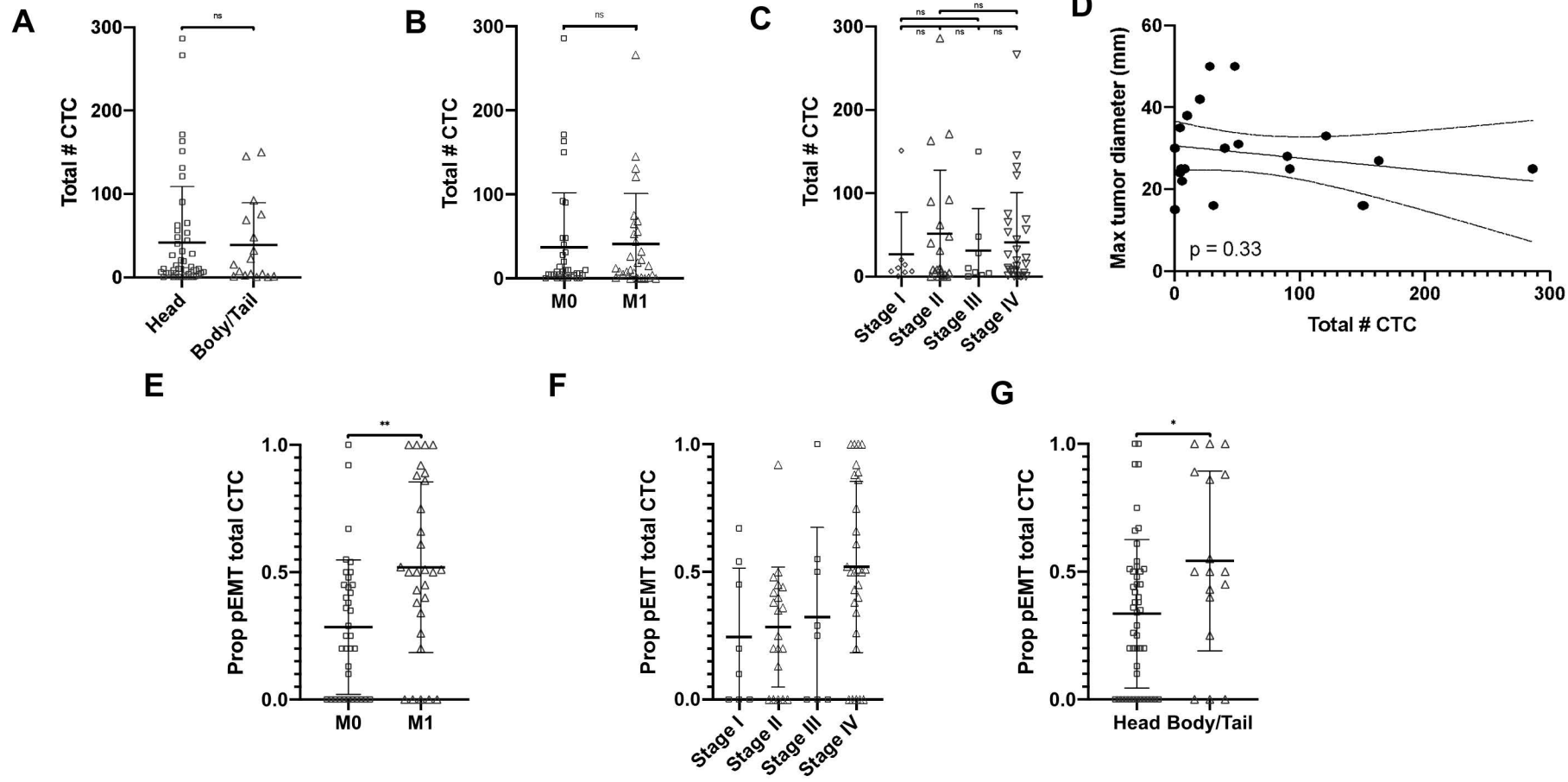
**Supplementary table 6:**

PFS and OS of different pEMT total counts in BTN draws (n=65)

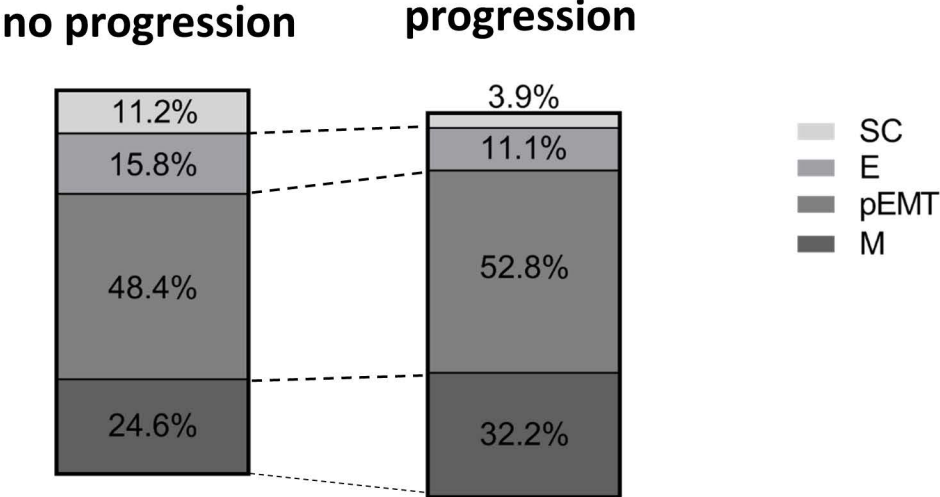
**Supplementary table 7:**

Comparison of different clinico-pathological parameters with CTC subtypes across last draw before surgery (n=22).

# Supplementary figure 1

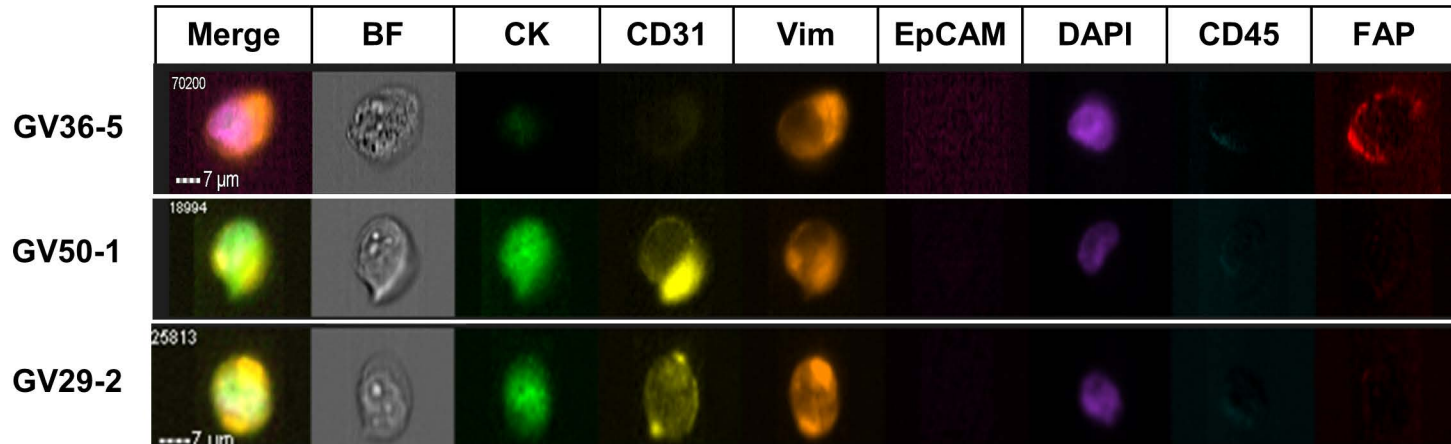


# Supplementary figure 2



# Supplementary figure 3

**A**



**B**

Sample	CD31+/CK+	FAP+/CK+	Vim+/CK+	CD45-
GV29-2	2*	3	15	179
GV50-1	2	1	21	103
GV36-5	0	1	23	108

**Supplementary Table 1:** Comparison of different clinico-pathological parameters with CTC subtypes in treatment-naïve patients (n=65).

Clinico-pathological Parameters	Total Number	Mean +/- std, median* Total CTC	P-value#	Mean +/- std, median* E-CTC	P-value#	Mean +/- std, median* pEMT-CTC	P-value#	Mean +/- std, median* M-CTC	P-value#	Mean +/- std, median* SC-CTC	P-value#	Mean +/- std, median* Prop-pEMT (%)	P-value#
BTN PDAC Cases 65 (100%)													
<b>Localization</b>													
Head	48 (73.8%)	41.94 +/- 66.99, 10 (5, 50.5)	0.9941	1.83 +/- 5.65, 0 (0, 1)	0.9099	20.54 +/- 34.16, 4 (0, 22)	0.5097	13.63 +/- 23.14, 4 (0.5, 13.5)	0.5190	5.94 +/- 19.95, 0 (0, 2)	0.5604	0.34 +/- 0.29, 0.35 (0, 0.5)	0.0472
Body/Tail	17 (26.2%)	39.21 +/- 50.19, 15 (2, 68.5)		0.62 +/- 1.5, 0 (0, 0.5)		22.47 +/- 28.83, 6 (1, 41)		14.41 +/- 24.51, 3 (0, 7)		1.71 +/- 3.44, 0 (0, 0)			
<b>Tumor size</b>													
0-4cm	15 (83.3%)	67.13 +/- 83.48, 31 (5, 121)	1.000	3.13 +/- 9.33, 0 (0, 1)	0.7679	34.8 +/- 41.77, 15 (1, 80)	0.9070	16.2 +/- 20.81, 5 (2, 22)	0.4855	13 +/- 33.4, 0 (0, 9)	0.5242	0.4 +/- 0.25, 0.44 (0.2, 0.54)	0.7267
>4cm	3 (16.7%)	32 +/- 14.42, 28 (20, 48)		4.33 +/- 7.51, 0 (0, 13)		9.67 +/- 2.08, 9 (8, 12)		17.33 +/- 16.2, 9 (7, 36)		0.67 +/- 1.15, 0 (0, 2)		0.33 +/- 0.11, 0.29 (0.25, 0.45)	
<b>Distant Metastasis (M)</b>													
<b>Category</b>													
M <sub>0</sub>	36 (55.4%)	41.22 +/- 65.76, 9 (4, 48)	0.6831	1.06 +/- 2.75, 0 (0, 0)	0.3520	18.58 +/- 31.97, 1.5 (0, 17.5)	0.1941	15.58 +/- 23.54, 5 (2, 21.5)	0.1386	6 +/- 22.06, 0 (0, 1.5)	0.8572	0.28 +/- 0.26, 0.25 (0, 0.45)	0.0046
M <sub>1</sub>	29 (44.6%)	41.22 +/- 59.75, 15 (4, 56)		2.09 +/- 6.74, 0 (0, 1)		24.1 +/- 33.75, 6 (1, 34)		11.66 +/- 23.26, 3 (0, 7)		3.38 +/- 8.39, 0 (0, 2)		0.52 +/- 0.34, 0.5 (0.34, 0.86)	
<b>Histopathological Grade</b>													
G <sub>2</sub> (moderate)	13 (68.4%)	73.85 +/- 85.46, 31 (10, 121)	0.2193	4.62 +/- 10.24, 0 (0, 2)	0.3782	37.46 +/- 43.42, 12 (3, 80)	0.2039	17.31 +/- 20.94, 8 (3, 22)	0.5177	14.46 +/- 35.77, 1 (0, 5)	0.2040	0.4 +/- 0.23, 0.4 (0.25, 0.48)	0.5462
G <sub>3</sub> (poor)	6 (31.6%)	25.5 +/- 35.56, 8 (5, 40)		0.83 +/- 2.04, 0 (0, 0)		10.83 +/- 16.24, 2.5 (1, 18)		12.33 +/- 16.56, 4 (2, 22)		1.5 +/- 3.67, 0 (0, 0)		0.31 +/- 0.25, 0.32 (0.1, 0.45)	
<b>UICC Stage</b>													
I	8 (12.3%)	26.5 +/- 50.68, 8 (5.5, 17)	0.9274	0.63 +/- 1.77, 0 (0, 0)	0.7000	12 +/- 28.05, 1 (0, 6.5)	0.4121	12 +/- 18.69, 5 (3, 11.5)	0.5064	1.88 +/- 4.55, 0 (0, 1)	0.2107	0.24 +/- 0.27, 0.15 (0, 0.49)	0.0315

II	20 (30.8%)	51.25 +/- 76.31, 9 (3, 76)	0.7 +/- 1.84, 0 (0, 0.5)	23.2 +/- 35.29, 2.5 (0.5, 33.5)	17.3 +/- 26.53, 5.5 (2, 22)	10.05 +/- 29.15, 0 (0, 7)	0.28 +/- 0.23, 0.3 (0.06, 0.43)
III	8 (12.3%)	30.88 +/- 50.86, 7.5 (3, 38)	2.38 +/- 4.78, 0 (0, 3)	13.63 +/- 28.38, 3 (0, 10)	14.88 +/- 22.08, 5.5 (0.5, 23)	0 +/- 0, 0 (0, 0)	0.32 +/- 0.35, 0.27 (0, 0.53)
IV	29 (44.6%)	41.22 +/- 59.75, 15 (4, 56)	2.09 +/- 6.74, 0 (0, 1)	24.1 +/- 33.75, 6 (1, 34)	11.66 +/- 23.26, 3 (0, 7)	3.38 +/- 8.39, 0 (0, 2)	0.52 +/- 0.34, 0.5 (0.34, 0.86)

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\*Interquartile

#Wilcoxon rank sum test p-value

**Supplementary table 2:** Comparison of different clinico-pathological parameters with CTC subtypes in all draws on therapy (n=132).

Response to therapy	Total Number of blood draws on therapy (n=132)	Mean +/- std, median* Total CTC	P-value#	Mean +/- std, median* E-CTC	P-value#	Mean +/- std, median* pEMT-CTC	P-value#	Mean +/- std, median* M-CTC	P-value#	Mean +/- std, median* SC-CTC	P-value#	Mean +/- std, median* Prop-pEMT (%)	P-value#
No progression	103 (78.0%)	16.94 +/- 32.16, 9 (0, 16)	0.0162	2.68 +/- 12.31, 0 (0, 1)	0.0489	8.19 +/- 17.86, 3 (0, 7)	0.0589	4.17 +/- 8.8, 1 (0, 6)	0.0087	1.89 +/- 4.11, 0 (0, 1)	0.5230	0.31 +/- 0.31, 0.33 (0, 0.5)	0.0410
Progression	29 (22.0%)	31.93 +/- 61.06, 15 (5, 22)		3.55 +/- 10.33, 0 (0, 2)		16.86 +/- 33.59, 3 (2, 20)		10.28 +/- 19.16, 5 (0, 10)		1.24 +/- 3.5, 0 (0, 1)		0.42 +/- 0.31, 0.49 (0.14, 0.6)	

\*Interquartile

#Wilcoxon rank sum test p-value



**Supplementary table 3:** Cox proportional hazard analysis of clinicopathological parameters. A) Overall survival of baseline treatment-naïve patients (n=65), B) Progression-free survival of baseline treatment-naïve patients (n=65) C) Multivariable Cox model for PFS.

A. OS in treatment-naïve patients

<b>Clinico-pathological parameter</b>	<b>Hazard ratio [95% CI] Log transformed</b>	<b>p-value</b>	<b>Total</b>	<b>Events</b>
Total CTC number	0.91(0.81,1.02)	0.092	65	23
E-CTC number	0.99(0.84,1.15)	0.852	65	23
pEMT-CTC number	0.93(0.83,1.04)	0.191	65	23
M-CTC number	0.9(0.79,1.02)	0.104	65	23
SC-CTC number	0.91(0.78,1.06)	0.215	65	23
pEMT-Proportion/total CTC	0.76(0.18,3.29)	0.712	65	23
Gender (m vs. f)	1.43(0.61,3.3)	0.408	65	23
Age	0.97(0.93,1.01)	0.105	65	23
Prop_EMT/total CTC (>50%)	0.92(0.36,2.34)	0.860	65	23

B. PFS in treatment-naive patients

<b>Clinico-pathological parameter</b>	<b>Hazard ratio [95% CI] Log transformed</b>	<b>p-value</b>	<b>Total</b>	<b>Events</b>
Total CTC number	0.97(0.89,1.07)	0.564	65	49
E-CTC number	0.99(0.88,1.1)	0.837	65	49
pEMT-CTC number	0.99(0.9,1.08)	0.748	65	49
M-CTC number	0.95(0.87,1.04)	0.300	65	49
SC-CTC number	0.92(0.83,1.02)	0.095	65	49
pEMT-Prop/total CTC	2.17(0.8,5.92)	0.130	65	49
Gender (m vs. f)	1.5(0.83,2.72)	0.180	65	49
Age	0.97(0.94,1)	0.058	65	49
pEMT-Prop/total CTC (>50%)	2.04(1.12,3.72)	0.020	65	49
Stage I/II vs. stage IV	0.37 (0.20, 0.70)	0.009	65	49
Stage III vs. stage IV	0.59 (0.24, 1.45)			

C. Multivariate Cox Model for PFS

<b>Clinico-pathological parameter</b>		<b>Hazard Ratio</b>	<b>95% Hazard Ratio Confidence Limits</b>		<b>p-value</b>
Stage	I/II vs. IV	0.54	0.27	1.06	0.190
	III vs IV	0.66	0.26	1.67	
SC-CTC number		0.90	0.81	1.01	0.066
Age		0.97	0.94	1.01	0.093
pEMT-Prop (>50%)		1.98	1.05	3.73	0.036

**Supplementary Table 4.** Summary of calculated PFS (days) using different proportional cutoffs for E, M, pEMT and SC in BTN draws (n=65)

Cell Type	Mean survival (days) with SD below cutoff	Mean survival (days) with SD above cutoff	p value (Log Rank)
<b>Prop_pE cutoff</b>			
0.7		only one case <sup>a</sup>	0.33
0.6		only one case <sup>a</sup>	0.33
0.5		only one case <sup>a</sup>	0.33
0.4		only three cases	0.085
0.3	335.6 ± 38.7	396.2 ± 76.9	0.15
0.2	341.4 ± 39.8	323.1 ± 75.4	0.41
0.1	340.4 ± 40.9	410.8 ± 102.3	0.34
<b>Prop_pM cutoff</b>			
0.7	353.4 ± 43.2	288.9 ± 54.9	0.91
0.6	259.3 ± 44.8	277.1 ± 47.1	0.68
0.5	358.9 ± 46.5	291.2 ± 45.1	0.78
0.4	315.7 ± 39.8	352.7 ± 57.9	0.98
0.3	287.4 ± 44.1	375.4 ± 53.1	0.44
0.2	274.2 ± 43.8	386.1 ± 53.7	0.25
0.1	280.3 ± 45.1	379.2 ± 52.7	0.34
<b>Prop_pSC cutoff</b>			
0.7	b	b	b
0.6	b	b	b
0.5	b	b	b
0.4	327.0 ± 37.6	503.0 ± 124.9	0.11
0.3	328.1 ± 38.3	443.2 ± 107.0	0.18
0.2	324.5 ± 38.6	408.8 ± 89.5	0.19
0.1	339.4 ± 40.8	318.2 ± 71.6	0.80
<b>Prop_pEMT cutoff</b>			
0.7	387.4 ± 45.1	183.1 ± 53.7	0.015
0.6	395.5 ± 46.6	195.1 ± 52.5	0.011
0.5	403.5 ± 49.5	216.4 ± 45.4	0.018
0.45	375.5 ± 42.7	266.8 ± 60.3	0.069
0.4	403.5 ± 49.5	341.4 ± 58.6	0.74
0.3	319.3 ± 38.1	329.5 ± 49.4	0.46
0.2	307.7 ± 41.5	345.7 ± 48.0	0.78
0.1	306.8 ± 49.8	349.1 ± 44.9	0.85

<sup>a</sup>No statistics are computed because all cases are censored

<sup>b</sup>No comparison analysis is performed because the factor variable has only one value for every stratum.

**Supplementary Table 5.** Summary of calculated OS (days) using different proportional cutoffs for E, M, pEMT and SC in BTN draws (n=65)

Cell type	Mean survival (days) with SD below cutoff	Mean survival (days) with SD above cutoff	p value (Log Rank)
<b>Prop_pE cutoff</b>			
0.7		only one case <sup>a</sup>	0.59
0.6		only one case <sup>a</sup>	0.59
0.5		only one case <sup>a</sup>	0.59
0.4		only three cases	0.26
0.3	652.6 ± 44.4	473.7 ± 38.3	0.71
0.2	654.8 ± 45.2	479.0 ± 34.4	0.89
0.1	644.6 ± 46.6	700.3 ± 81.2	0.56
<b>Prop_pM cutoff</b>			
0.7	655.9 ± 46.4	509.0 ± 51.4	0.88
0.6	644.2 ± 47.2	533.8 ± 46.6	0.68
0.5	643.1 ± 48.1	529.7 ± 42.9	0.71
0.4	601.1 ± 48.9	690.3 ± 60.8	0.79
0.3	514.5 ± 35.9	724.1 ± 51.5	0.16
0.2	506.3 ± 36.8	730.2 ± 50.3	0.11
0.1	515.5 ± 37.5	719.3 ± 50.1	0.19
<b>Prop_pSC cutoff</b>			
0.7	<sup>b</sup>	<sup>b</sup>	<sup>b</sup>
0.6	<sup>b</sup>	<sup>b</sup>	<sup>b</sup>
0.5	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>
0.4	598.0 ± 48.3	678.2 ± 60.9	0.55
0.3	645.6 ± 45.8	602.0 ± 75.9	0.49
0.2	645.6 ± 46.7	584.6 ± 63.9	0.57
0.1	653.4 ± 48.0	547.2 ± 43.4	0.97
<b>Prop_pEMT cutoff</b>			
0.7	684.7 ± 44.0	498.4 ± 78.3	0.39
0.6	682.9 ± 45.6	560.7 ± 80.9	0.59
0.5	668.9 ± 48.9	617.9 ± 65.1	0.86
0.45	638.4 ± 43.1	630.8 ± 69.7	0.68
0.4	598.0 ± 48.3	678.2 ± 60.9	0.55
0.3	498.5 ± 31.2	688.6 ± 54.1	0.33
0.2	493.1 ± 34.2	693.2 ± 51.7	0.23
0.1	496.5 ± 37.9	686.2 ± 49.8	0.31

<sup>a</sup>No statistics are computed because all cases are censored

<sup>b</sup>No comparison analysis is performed because the factor variable has only one value for every stratum.

<sup>c</sup>No statistics are computed because all cases are censored.

**Supplementary Table 6:** PFS and OS (days) of different pEMT total counts in BTN draws (n=65)

	<b>total_pEMT cutoff</b>	<b>Mean survival (days) with SD below cutoff</b>	<b>Mean survival (days) with SD above cutoff</b>	<b>p value (Log Rank)</b>
<b>PFS</b>				
	75	668.9 ± 45.0	607.7 ± 59.4	0.92
	50	662.5 ± 48.1	575.5 ± 53.1	0.87
	30	644.4 ± 50.4	656.1 ± 69.5	0.44
	20	647.9 ± 51.6642.6 ± 65.6	266.8 ± 60.3	0.51
	15	645.8 ± 53.7	633.9 ± 63.5	0.58
	10	574.5 ± 48.3	722.5 ± 62.4	0.13
	5	483.3 ± 29.9	722.9 ± 55.1	0.09
	1	488.8 ± 39.6	690.7 ± 49.2	0.21
<b>OS</b>				
	75	340.6 ± 40.5	373.7 ± 112.5	0.45
	50	355.5 ± 44.0	296.5 ± 66.2	0.82
	30	357.2 ± 46.2	303.5 ± 57.9	0.82
	20	366.5 ± 49.2	290.4 ± 48.9	0.56
	15	375.0 ± 51.7	289.4 ± 46.0	0.46
	10	325.0 ± 44.8	360.0 ± 60.1	0.84
	5	276.0 ± 34.9	377.9 ± 55.2	0.47
	1	289.6 ± 50.2	358.1 ± 45.3	0.83

**Supplementary table 7:** Comparison of different clinico-pathological parameters with CTC subtypes across last draw before surgery (n=22).

Recurrence after surgery	Total Number (n=22)	Mean +/- std, median* Total CTC	P-value#	Mean +/- std, median* E-CTC	P-value#	Mean +/- std, median* pEMT-CTC	P-value#	Mean +/- std, median* M-CTC	P-value#	Mean +/- std, median* SC-CTC	P-value#	Mean +/- std, median* Prop-pEMT (%)	P-value#
>12 month	15 (68.2%)	42.67 +/- 74.6, 14 (5, 31)	0.6020	7.2 +/- 15.47, 0 (0, 8)	0.6488	16.53 +/- 31.97, 6 (1, 15)	0.3169	8.73 +/- 12.93, 3 (1, 13)	0.9159	10.2 +/- 32.98, 1 (0, 3)	0.5401	0.32 +/- 0.19, 0.36 (0.2, 0.44)	0.2436
< 12 month	7 (31.8%)	43 +/- 46.05, 20 (12, 92)		5.71 +/- 13.4, 0 (0, 3)		23.71 +/- 28.06, 14 (4, 41)		10.29 +/- 15.39, 5 (0, 18)		3.29 +/- 3.68, 1 (0, 7)		0.49 +/- 0.3, 0.45 (0.33, 0.7)	

\*Interquartile

#Wilcoxon rank sum test p-value