

Supplementary Table S1

Antibodies dilution and acquisition parameters used for ImFC.

Antibody	Company	Cat. nr.	Fluorophor	Dilution	Laser	Laser power	Channel
BF							1+9
pan-keratin (AE1/AE3)	Thermo Fisher Scientific	53-9003-82	Alexa 488	1:2500	488	100mW	2
Pan-keratin (C11)	Thermo Fisher Scientific	MA5-18156	Alexa 488	1:2500	488	100mW	2
a-SMA (1A4)	R&Dsystems	IC1420P	PE	1:50	488	100mW	3
SSC					785		6
DAPI	BD Biosciences	564907	DAPI	0,04ug/ml	405	100mW	7
CD29 (TS2/16)	Thermo Fisher Scientific	63-0299-42	SuperBright600	1:1000	405	100mW	10
Vimentin (D21H3)	Cell Signaling	9856	Alexa 647	1:100	647	150mW	11
CD45 (REA747)	Miltenyi Biotec	130-110-635	APC-Vio770	1:50	647	150mW	12
CD31 (WM59)	BioLegend	303120	APC-Cy7	1:10	647	150mW	12

Supplemental Table S2

Correlations of CTC phenotypes with cCAFs.

		epi CTCs	epi-mes CTCs	mes CTCs	neg CTCs	cCAFs
epi CTCs	R ² p-values n	1 210	-0.015 0.813 210	0.259 <0.001 210	0.772 <0.001 210	0.165 0.017 210
epi-mes CTCs	R ² p-values n		1 210	0.086 0.215 210	0.105 0.128 210	0.086 0.214 210
mes CTCs	R ² p-values n			1 210	0.314 <0.001 210	0.097 0.163 210
neg CTCs	R ² p-values n				1 210	0.250 <0.001 210
cCAFs	R ² p-values n					1 210

Supplementary Table S3

Comparison of exclusive CTC phenotypes among clinico-pathological features and response to treatment of patients with breast cancer. Chi squared test was performed.

Due to missing data not all numbers sum up to 210.

		Exclusive CTC phenotypes					
Variable	total n	epi	mes	epi-mes	neg	heterogeno us	p-value
Age							
<50	16	2 (18.2%)	2 (12.5%)	2 (66.7%)	0 (0%)	10 (43.5%)	0.02
>>50	42	9 (81.8%)	14 (87.5%)	1 (33.3%)	5 (100%)	13 (56.5%)	
cT stage							
cT1-2	27	5 (62.5%)	8 (72.7%)	2 (100%)	2 (50%)	10 (66.7%)	0.8
cT3-4	13	3 (37.5%)	3 (27.3%)	0 (0%)	2 (50%)	5 (33.3%)	
cN stage							
0	15	5 (62.5%)	4 (40%)	2 (100%)	0 (0%)	4 (25%)	0.11
1	25	3 (37.5%)	6 (60%)	0 (0%)	4 (100%)	12 (75%)	
M stage							
0	46	7 (63.6%)	12 (75%)	3 (100%)	5 (100%)	19 (82.6%)	0.05
1	12	4 (36.4%)	4 (25%)	0 (0%)	0 (0%)	4 (15.4%)	
Grading							
1	3	1 (11.1%)	1 (8.3%)	0 (0%)	0 (0%)	1 (5%)	0.46
2	26	3 (33.3%)	7 (58.3%)	3 (100%)	4 (100%)	9 (45 %)	
3	19	5 (55.6%)	4 (33.3%)	0 (0%)	0 (0%)	10 (50%)	
Molecular type							
Luminal A	9	1 (10%)	4 (25%)	1 (33.3%)	1 (25%)	2 (8.7%)	0.1
Luminal B HER2-	14	1 (10%)	4 (25%)	2 (66.7%)	0 (0%)	7 (30.4%)	
Luminal B HER2+	13	2 (20%)	4 (25%)	0 (0%)	3 (75%)	4 (17.4%)	
Non luminal HER2+	3	0 (0%)	1 (6.3%)	0 (0%)	0 (0%)	2 (8.7%)	
Triple Negative	17	6 (60%)	3 (18.8%)	0 (0%)	0 (0%)	8 (34.8%)	
Response to treatment							
stable disease	6	0 (0%)	2 (25%)	1 (100%)	2 (50%)	1 (8.3%)	0.45
complete response	9	2 (50%)	1 (12.5%)	0 (0%)	1 (25%)	5 (41.7%)	
partial response	13	2 (50%)	5 (62.5%)	0 (0%)	1 (25%)	5 (41.7%)	
progressive disease	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (8.3%)	

Supplementary Table S4

Distribution of dominant CTC phenotypes among clinico-pathological features and response to treatment of patients with breast cancer. Chi squared test was performed.

Due to missing data not all numbers sum up to 210.

Variable	total n	Dominant CTC phenotypes					p-value
		epi	mes	epi-mes	neg	heterogenous	
Age							
<50	16	6 (27.3%)	3 (17.6%)	4 (66.7%)	2 (20%)	1 (33.3%)	0.08
>>50	42	16 (73.7%)	14 (82.4%)	2 (33.3%)	8 (80%)	2 (66.7%)	
cT stage							
cT1-2	27	10 (71.4%)	8 (66.7%)	3 (100%)	4 (50%)	2 (66.7%)	0.65
cT3-4	13	4 (28.6%)	4 (33.3%)	0 (0%)	4 (50%)	1 (33.3%)	
cN stage							
0	15	6 (40%)	4 (36.4%)	2 (66.7%)	2 (25%)	1 (33.3%)	0.88
1	25	9 (60%)	7 (63.6%)	1 (33.3%)	6 (75%)	2 (66.7%)	
M stage							
0	46	16 (72.7%)	13 (76.5%)	4 (66.7%)	10 (100%)	3 (100%)	0.03
1	12	6 (23.1%)	4 (23.5%)	2 (33.3%)	0 (0%)	0 (0%)	
Grading							
1	3	2 (11.1%)	1 (7.7%)	0 (0%)	0 (0%)	0 (0%)	0.84
2	26	8 (44.4%)	7 (53.8%)	4 (80%)	6 (66.7%)	1 (33.3%)	
3	19	8 (44.4%)	5 (38.5%)	1 (20%)	3 (33.3%)	2 (66.7%)	
Molecular type							
Luminal A	9	2 (9.5%)	4 (23.5%)	1 (16.7%)	2 (22.2%)	0 (0%)	0.17
Luminal B HER2-	14	4 (19%)	4 (23.5%)	3 (50%)	1 (11.1%)	2 (66.7%)	
Luminal B HER2+	13	3 (14.3%)	4 (23.5%)	2 (33.3%)	4 (44.4%)	0 (0%)	
Non luminal HER2+	3	1 (4.8%)	1 (5.9%)	0 (0%)	1 (11.1%)	0 (0%)	
Triple Negative	17	11 (52.4%)	4 (23.5%)	0 (0%)	1 (11.1%)	1 (33.3%)	
Response to treatment							
stable disease	6	0 (0%)	2 (25%)	2 (100%)	2 (28.6%)	0 (0%)	0.19
complete response	9	4 (44.4%)	1 (12.5%)	0 (0%)	3 (42.9%)	1 (33.3%)	
partial response	13	4 (44.4%)	5 (62.5%)	0 (0%)	2 (28.6%)	2 (66.7%)	
progressive disease	1	1 (11.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	