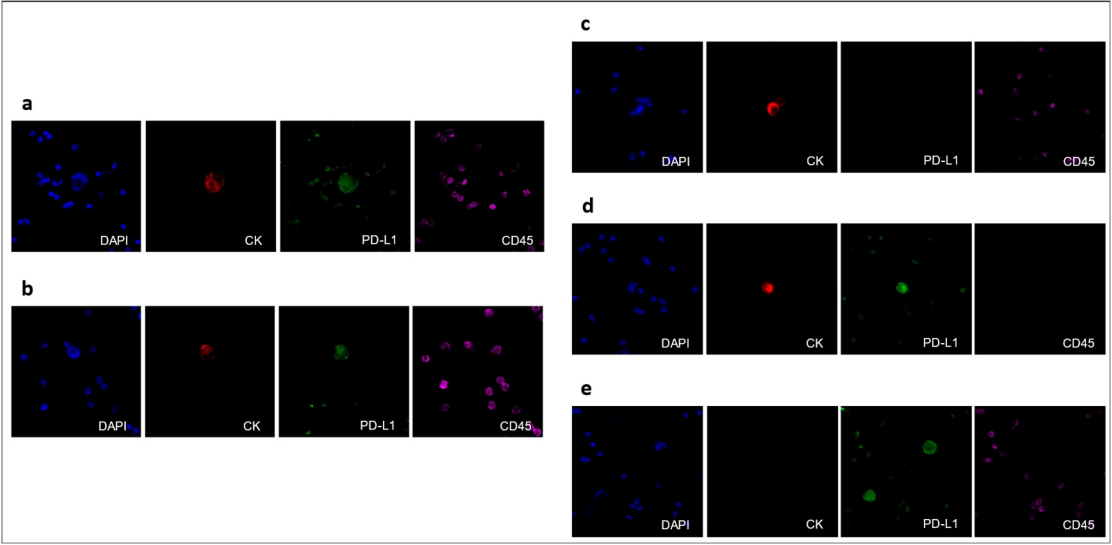
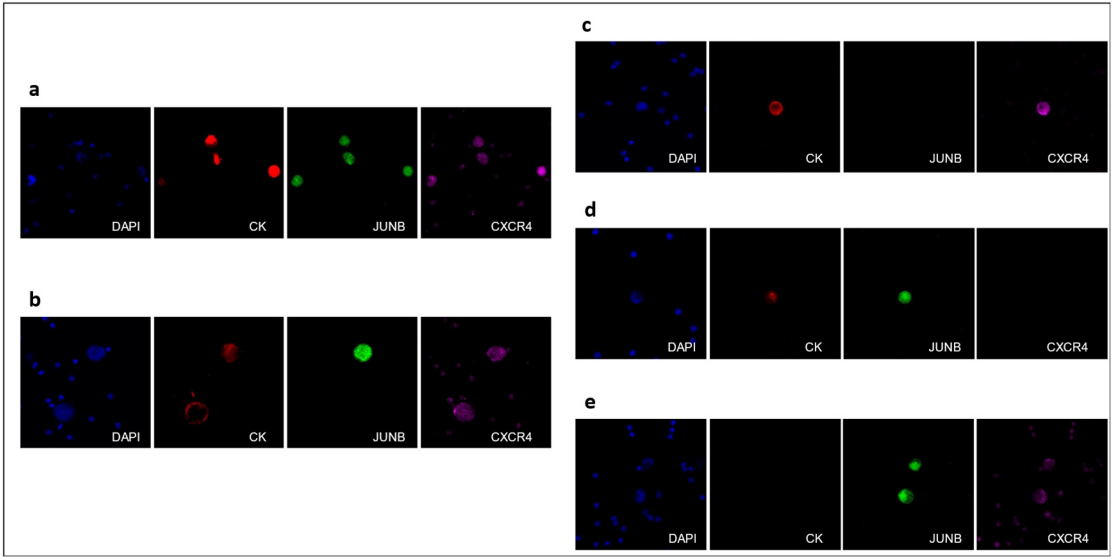


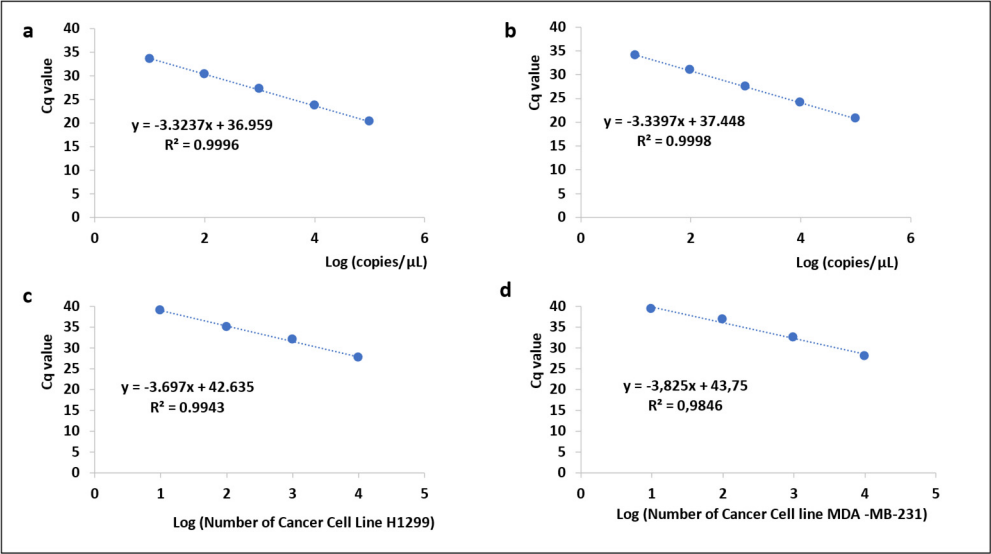
Supplementary Figure S1



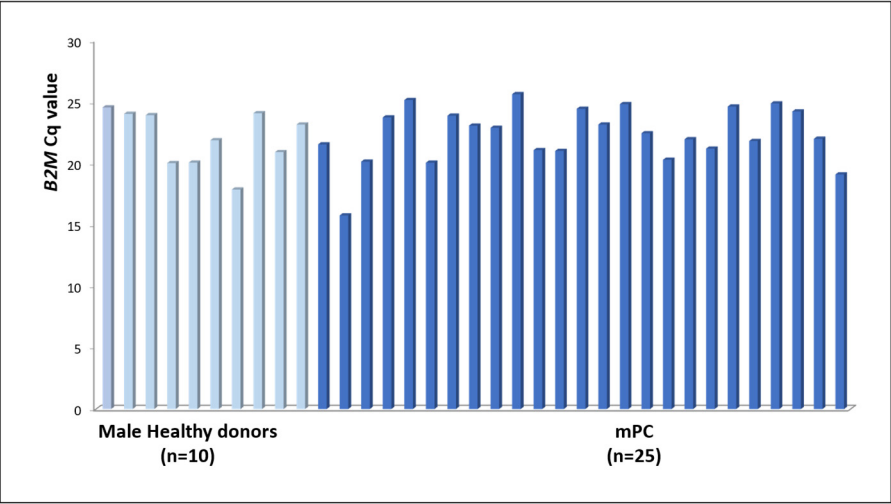
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5a



Supplementary Figure S5b

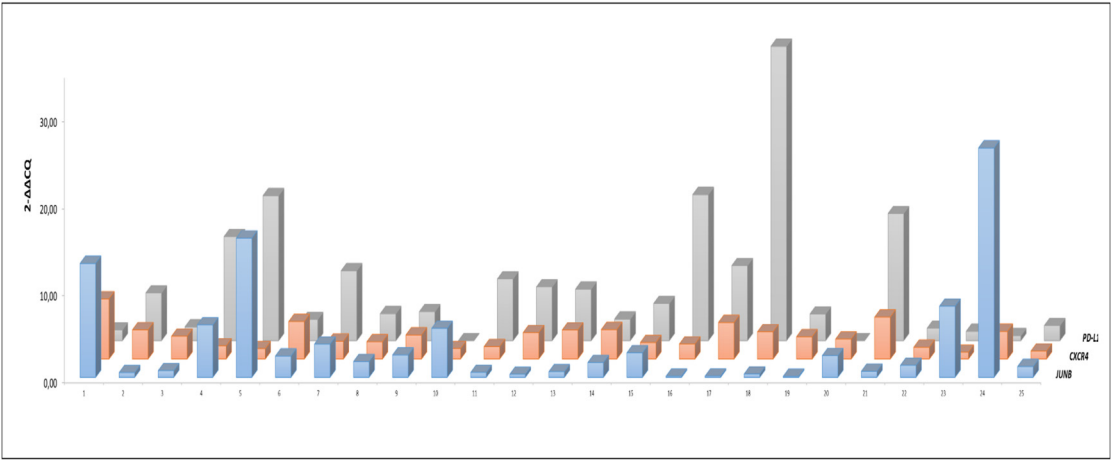


Table S1. Patients' characteristics enrolled in Ficoll density isolation (48 patients).

Variable	Sub-categories n (%)
Age	
≥ 75	20 (41.7 %)
< 75	13 (27.1 %)
Unknown	15 (31.2 %)

Gleason score, n (%)	
≥ 8	24 (50 %)
< 8	14 (29.2 %)
Unknown	10 (20.8 %)
Mean PSA (Range), (ng/mL)	
	89.9 (1.6-342)
Smoking Consumption	
Yes	13 (27.1 %)
No	27 (56.2 %)
Unknown	8 (16.7 %)
Disease stage	
Metastatic	48 (100.0 %)
Metastasis locations	
Bone metastasis	19 (39.6 %)
Lymph node metastasis	12 (25.0 %)
Lung metastasis	4 (8.33 %)
Bone marrow metastasis	1 (2.1 %)
Kidney metastasis	1 (2.1 %)
Unknown	11 (22.9 %)
Chemotherapy	
Baseline	38 (79.2 %)
After 1 st line	10 (20.8 %)

Table S2. Patients' characteristics enrolled in mRNA analysis (25 patients).

Variable	Sub-categories n (%)
Age	
≥ 75	8 (32.0 %)
< 75	8 (32.0 %)
Unknown	9 (36.0 %)
Gleason score, n (%)	
≥ 8	12 (48.0 %)

< 8	7 (28.0 %)
Unknown	6 (24.0 %)
Mean PSA (Range), (ng/mL)	65.4 (2-251)
Smoking Consumption	
Yes	4 (16.0 %)
No	16 (64.0 %)
Unknown	5 (20.0 %)
Disease stage	
Metastatic	25 (100.0 %)
Metastasis locations	
Bone metastasis	8 (32.0 %)
Lymph node metastasis	9 (36.0 %)
Lung metastasis	1 (4.0 %)
Bone marrow metastasis	1 (4.0 %)
Kidney metastasis	1 (4.0 %)
Unknown	5 (20.0 %)
Chemotherapy	
Baseline	25 (100.0 %)
After 1 st line	0 (0 %)

Table S3. Patients' characteristics enrolled in ISET isolation (17 patients).

Variable	Sub-categories n (%)
Age	
≥ 75	7 (41.2 %)
< 75	8 (47.0 %)
Unknown	2 (11.8 %)
Gleason score, n (%)	
≥ 8	6 (35.3 %)
< 8	5 (29.4 %)
Unknown	6 (35.3 %)

Mean PSA (Range), (ng/mL)	95.2 (7.9-251)
Smoking Consumption	
Yes	6 (35.3 %)
No	7 (41.2 %)
Unknown	4 (23.5 %)
Disease stage	
Metastatic	17 (100.0 %)
Metastasis locations	
Bone metastasis	9 (52.9 %)
Lymph node metastasis	6 (35.3 %)
Lung metastasis	2 (11.8%)
Bone marrow metastasis	0 (0 %)
Kidney metastasis	0 (0 %)
Unknown	0 (0 %)
Chemotherapy	
Baseline	17 (100.0 %)
After 1 st line	0 (0 %)

Table S4. CTCs per patient after ficoll density gradient isolation and triple immunofluorescent staining CK/PD-L1/CD45.

Patient ID#number	Total CK+ cells (CTCs)	CK+/PD-L1+/CD45-	CK+/PD-L1-/CD45-
1	0	0	0
2	1	1	0
3	0	0	0
4	1	0	1
5	5	5	0
6	6	0	6
7	1	1	0
8	2	2	0
9	0	0	0

10	0	0	0
11	0	0	0
12	0	0	0
13	0	0	0
14	0	0	0
15	0	0	0
16	1	1	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0
21	1	0	1
22	1	1	0
23	2	2	0
24	1	1	0
25	3	3	0
26	0	0	0
27	1	1	0
28	2	0	2
29	0	0	0
30	0	0	0
31	0	0	0
32	0	0	0
33	0	0	0
34	0	0	0
35	0	0	0
36	0	0	0
37	0	0	0
38	0	0	0
39	0	0	0
40	1	1	0
41	0	0	0
42	1	0	1
43	1	0	1
44	0	0	0
45	1	0	1
46	0	0	0

47	0	0	0
48	0	0	0

Table S5. Wilcoxon signed-rank test results for the identified phenotypes from triple immunofluorescence stainings.

	CK+/PD-L1+/CD45- vs CK+/PD-L1-/CD45-	ISET_CK+/PD-L1+/CD45- vs ISET_CK+/PD-L1-/CD45-	CK+/CXCR4+ vs CK+/JUNB+	CK+/CX CR4+/JU NB+ vs CK+/CX CR4-/JUNB+	CK+/CX CR4+/JU NB+ vs CK+/CX CR4+/JU NB-	CK+/CX CR4+/JU NB+ vs CK+/CX CR4-/JUNB-	CK+/CX CR4+/JU NB- vs CK+/CX CR4-/JUNB+	CK+/CX CR4-/JUNB+ vs CK+/CX CR4-/JUNB-
Asymp. Sig. (2-tailed)	0.343	0.861	0.518	0.013	0.083	0.077	0.518	0.336

Table S6. CTCs per patients after ISET isolation and triple immunofluorescent staining CK/PD-L1/CD45.

Patient ID#number	Total CK+ cells (CTCs)	CK+/PD-L1+/CD45-	CK+/PD-L1-/CD45-
5	2	1	1
6	1	1	0
7	3	1	2
8	97	0	97
9	1	1	0
10	0	0	0
11	0	0	0
12	1	1	0
13	0	0	0
14	0	0	0
16	0	0	0
22	0	0	0
23	14	8	6
24	1	0	1
26	0	0	0
36	0	0	0
41	0	0	0

Table S7. CTCs per patient after ficoll density gradient isolation and triple immunofluorescent staining CK/CXCR4/JUNB.

Patient ID#number	Total CK+ cells (CTCs)	CK+/CXCR 4+/JUNB+	CK+/CXCR 4-/JUNB+	CK+/CXCR 4+/JUNB-	CK+/CXCR 4-/JUNB-
1	0	0	0	0	0
2	1	1	0	0	0
3	0	0	0	0	0
4	3	0	0	0	3
5	0	0	0	0	0
6	16	8	3	2	3
7	5	2	0	3	0
8	0	0	0	0	0
9	1	1	0	0	0
10	0	0	0	0	0
11	2	0	0	2	0
12	0	0	0	0	0
13	1	1	0	0	0
14	0	0	0	0	0
15	0	0	0	0	0
16	2	2	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
21	0	0	0	0	0
22	1	0	0	1	0
23	0	0	0	0	0
24	1	1	0	0	0
25	2	2	0	0	0
26	0	0	0	0	0
27	0	0	0	0	0
28	4	2	0	0	2
29	0	0	0	0	0
30	1	1	0	0	0
31	0	0	0	0	0

32	0	0	0	0	0
33	1	0	1	0	0
34	0	0	0	0	0
35	0	0	0	0	0
36	0	0	0	0	0
37	0	0	0	0	0
38	0	0	0	0	0
39	0	0	0	0	0
40	1	0	0	0	1
41	1	0	1	0	0
42	0	0	0	0	0
43	0	0	0	0	0
44	0	0	0	0	0
45	0	0	0	0	0
46	0	0	0	0	0
47	0	0	0	0	0
48	0	0	0	0	0