Supporting information for:

Phenotypic Profiling of Circulating Tumor Cells in Metastatic Prostate Cancer Patients Using Nanoparticle-Mediated Ranking

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Table of Contents:

Materials and Methods

Cell Culture. VCaP cells (ATCC, US) were cultured in DMEM medium (Sigma Aldrich, US) supplemented with 10% FBS. Human prostate cancer cells, PC3 and LnCaP were obtained from Dr. Alison Allan, London Health Sciences Centre, London, ON. PC3 cells (ATCC, US) were cultured in F12K media (Gibco, US) supplemented with 10% FBS. LnCaP cells and DU145 cells (ATCC, US) were cultured in RPMI media (Gibco, US) supplemented with 10% FBS. Cells were grown at 37^{o}C and 5% CO_2.

Device Fabrication. Microchips were fabricated by rapid prototyping using poly(dimethylsiloxane) (PDMS) soft-lithography starting with an SU-8 master on a silicon wafer (University Wafer, US). A PDMS (Dow Chemical, US) replica of the master was formed. After peeling the replica, holes were pierced for tubing connections. Bonding was enhanced and made irreversible by oxidizing both the replica and the cover in a plasma discharge for 1 minute prior to bonding. Silicone tubing was then added at the inlet and the outlet of the PDMS device. The channel depth was 100 μm. PDMS chips were conditioned with Pluronic F68 Sigma (St. Louis, US) to reduce sample adsorption and washed with PBS pH=7.4 before use using a syringe pump (Chemyx, US). Two arrays of NdFeB N52 magnets (KJ Magnetics, US),1.5 mm diameter and 8 mm long, were placed on both the bottom and top surfaces of the capture zones in the chip for the duration of the cell capture process.

CTC Capture and Preparation. CTC analysis was performed using cells in PBS buffer, cells spiked in healthy donor blood, and CTCs were obtained from patient samples. For cell culture analysis, 100 cells were introduced into 1% BSA in PBS buffer for analysis in the microfluidic device.

Patients (n=36) were recruited from the Princess Margaret Hospital according to the University's Research Ethics Board approved protocol. Patients received enzalutamide (160 mg/day) or abiraterone (1000 mg/day). 20ml of blood were collected in two CellSave

preservation tubes that contained anticoagulant EDTA (Johnson and Johnson, US). One tube of blood was shipped to the London Regional Cancer Program at the London Health Sciences Centre, Ontario, Canada, for CellSearch analysis, and the second tube was analysed using the microfluidic device. All blood samples were analyzed within 24 hours from sample collection. 10µl of anti-EpCAM Nano-Beads (MACS, US) were added to 1ml of blood/ or cell-suspension and incubated and mixed for 30 minutes at room temperature. N-cadherin- conjugated nanobeads were prepared by incubating anti-N-cadherin (0.5 mg/ml) (Abcam, US) and anti-biotin nanobeads (MACS, US) with cells or 1ml of blood for 30 minutes at room temperature. During this incubation time, the magnetic nanobeads attach to cells. Microfluidic devices were perfused with pluronic F68, and washed with PBS. In the case where we analyzed cell lines, 100 cells per chip were prepared in 1% BSA in PBS. Samples were introduced into the microfluidic device at 600µl/h using a syringe pump. Next 200 µl PBS-EDTA at 600µl/h was introduced to remove non-target cells. After this step, chips were immunostained as detailed below.

CTC Capture Efficiency. CTC capture efficiency is quantified for cells in PBS spiked in whole blood and captured in the microfluidic device or using CellSearch. 20 cells were spiked into 1ml of healthy blood.

$$Capture\ Efficiency = \frac{\text{Number of Cells Counted}}{\text{Number of Cells Spiked into Blood}}$$

On-Chip Immunostaining. After processing the blood, cells were fixed with 4% paraformaldehyde, and subsequently permeabilized with 0.2% Triton X-100 (Sigma-Aldrich, US) in PBS. Cells were immunostained with primary antibodies (1:50 dilution): biotin monoclonal anti-Cytokeratin 18 (Lifespan, US), biotin monoclonal anti-N-cadherin (Abcam, US), Androgen Receptor Alexa Fluor 555 (Cell Signaling, US), ARV7 Alexa Fluor 555 (Precision Antibody, US), CD45- APC (MACS, US) followed by secondary antibody Yellow-Avidin nanobeads (Invitrogen, US) (1:2500 dilution) to visualize the Ck- and NCadherin-

CTCs. All of the antibodies were prepared in 100 µl PBS plus 1% BSA and chips were stained for 30 minutes at a flow rate of 200µl/h. Chips were washed between each staining step using 200 µl 0.1% Triton X-100 in PBS, at 600µl/h for 10 minutes. Nuclei were stained with 100 µl DAPI ProLong Gold reagent (Invitrogen, US) at 600µl/h. After completion of staining, all devices were washed with PBS and stored at 4°C before scanning.

CTCs were detected from the same blood sample using different microfluidic devices. In the first device, CTCs were captured with EpCAM-MNPs and identified using immunofluorescence as $DAPI^+/CK^+/CD45^-$. In the second device, CTCs were captured with EpCAM-MNPs and identified as $DAPI^+/CK^+/AR^{+/-}/CD45^-$. In the third device, CTCs were captured with EpCAM-MNPs and identified as $DAPI^+/CK^+/ARV7^{+/-}/CD45^-$. In the fourth device, CTCs were captured with EpCAM-MNPs and identified as $DAPI^+/NCad^+/CD45^-$. In the fifth device, CTCs were captured with NCad-MNPs and identified as $DAPI^+/CK^+/CD45^-$.

Image Scanning and Analysis. After immunostaining, the devices were scanned using a 10X and 50X objective and a fluorescent Nikon TiE eclipse microscope with an automated stage controller and an Andor camera (Nikon, US). Bright field as well as DAPI, FITC, TRITC, and Cy5 channels were acquired with NIS Elements (Nikon, US), and target cells were counted. ARV7 and cytokeratin intensity analysis were performed using ImageJ. Background intensity values were subtracted from the measured values.

Flow Cytometry. Cells were harvested from tissue culture using 0.25% trypsin/EDTA (Sigma-Aldrich, US) and incubated with blocking buffer (PBS + 1% BSA) for 30 minutes. For each cell line, 5×10^5 cancer cells were fixed and permeabilized using 4% paraformaldehyde (Sigma-Aldrich, US) and 0.2% Triton X (Sigma-Aldrich, US) in PBS, respectively. Cells were then washed with PBS and suspended in PBS containing 1% BSA and 0.1% Tween20, and incubated with anti-EpCAM Alexa Fluor 647 (BioLegend, US), N-cadherin- FITC (Cell Signaling, US), Androgen Receptor Alexa Fluor 647 (Cell Signaling, US) and ARV7 Alexa

Fluor 647 (Precision Antibody, US) at 1:50 dilution and stained at room temperature for 30 minutes. Samples were washed with PBS and re-suspended in PBS. Samples were injected into a BD FACS Canto flow cytometer (BD Biosciences, US) and measurements were plotted as histograms or median fluorescence for each fluorophore (AF647 and FITC). Median fluorescence values were normalized to an unstained control. A total of 5,000-10,000 cells were analyzed per cell line.

Progressive vs. Responsive Categorization. mCRPC patients were categorized as PSA-progressive or PSA- responsive according to PCWG3 criteria.[1] PSA response is defined as a >50% decline from baseline measured twice 3 to 4 weeks apart.

PSA progression is defined as, after decline from baseline, the time from initiation of therapy to the first PSA increase that is ≥ 25% and ≥ 2 ng/mL above the nadir, and which is confirmed by a second value 3 or more weeks later (ie, a confirmed rising trend). If there is no decline from baseline, PSA progression is defined as an increase of ≥ 25% and ≥ 2ng/mL from baseline, sustained after 12 weeks.

Statistics. Statistics were performed with two-sampled t-test for normally distributed populations and Mann Whitney test for non-parametric samples. $p<0.05$ is considered statistically significant.

Table S1. Prior drug treatment for mCRPC patients. Percentages indicate the proportion of treatments given relative to the total number of treatments recorded per patient. CTC data is obtained from 36 prostate cancer patients.

LHRH agonists (46%)	Anti-Androgens (47%)	Steroids (5%)	Immune Therapy (2%)
Triptorelin (Trelstar)	Bicalutamide (Casodex)	Prednisone	Prostvac Trial
Leuprolide (Eligard)	Nilutamide		
Goserelin (Zoladex)	Apalutamide		
Degarelix (Firmagon)			

Table S2. Metastatic sites, Gleason score and tumor stage for responsive mCRPC patients.

Patient ID	Total GS	Tumor stage	Radiological Evidence Type	Metastases location
1	6	PT2a	CT, bone scan	Lymph nodes, thoracic spine
2	7	PT2b	CT	Lymph nodes
3	7	N/A	CT	Lymph nodes
4	N/A	N/A	CT, bone scan	Diffuse osseous, left iliac chain lymph node. skull, sternum, left lower jaw, left clavicle, both scapulae, both humeri, most ribs, cervical, dorsal, lumbar, sacral spine, both sides of the pelvis, both femurs. Lung (micronodule in left lower lobe)
5	N/A	unknown	CT	T12 epidural mass; iliac bone
6	8	T3b	CT	T11, right hemipelvis, sacrum, lumbar spine, right proximal femur
7	8	N/A	CT	Lymph nodes
8	7	T3A	Bone scan	rib, sacrum
9	7	PT3b	CT	Lymph nodes
10	7	PT3	CT, bone scan	right ilium;L1;L5; left ilium; left acetabulum
11	6	T3c	CT, bone scan	left scapula, left ilium, right proximal femur. manubrium sternum, left 6th rib, left superior and inferior pubic rami. local recurrence: soft tissue nodule posterior to left bladder neck
12	9	PT3b	CT	S1;S2
13	7	T2 (left base nodule on DRE)	CT	bilateral lungs
14	7	N/A	CT, bone scan	suspicious lymph node, Left antesuperior iliac spine
15	unknown	unknown	Bone scan	Bone- 11th rib
16	9	N/A	CT	L2;L3; bilat iliac bones; sacrum; right acetabulum

Table S3. Metastatic sites, Gleason score and tumor stage for progressive mCRPC patients.

Patient ID	Total GS	Tumor stage	Radiological Evidence Type	Metastases location
17	7	PT3a	CT, bone scan	upper thoracic spine, L2,L5, Left 10th rib, 8th rib, left SI joint. All lumbar vertebraw, sacrum and both iliac wings
18	6	PT3a	CT	Lymph nodes, left 6th rib
19	7	T2 (nodule felt on DRE)	CT	Retroperitoneal lymph nodes
20	6	unknown	Bone scan	Bone scan: Left 10th and Right 9th ribs, CT: suspicious lymph nodes
21	N/A	N/A	Bone scan	throughout axial skeleton, proximal humerus, several ribs, sternum, throughout the thoracic and lumber spine and in the pelvis
22	7	PT3a	CT, bone scan	Iliac bones, sacrum, L5 and right L2, left scapula, T10 and left ilium
23	8	unknown	CT	Lymph nodes
24	7	PT2	Bone Scan	L1, L3, iliac crest (suspicious), left SI joint (suspicious)
25	7	unknown	FNA	Lymph node: Supracalvicular lymph node
26	9	PT2c	CT	Retroperitoneal Lymph nodes
27	7	unknown	CT, bone scan	sacrum, iliac bones, lymph nodes, L5, right mid-femur
28	9	T3b	Bone scan+MRI	left iliac bone
29	7	PT3b	Bone scan	Iliac Crest, proximal humerus
30	7	T2N1	biopsy, CT	Lymph node, Scapula (indeterminate sclerotic focus)
31	9	unknown	MRI, bone scan	left ischium, left anterior 4th rib
32	7	T2C-early T3	Bone scan	T3, T5, T11, T12, Right scapular spine, right 9th rib, left 9th, 10th rib, sacrum, right iliac bone. Calvarium, left 7th rib, L5-S1, L3, left sternoclavicular junction, left pubic ramus
33	unknown	unknown	CT	Sacrum
34	9	unknown	CT, bone scan	supra acetabular region; left proximal femur. Left iliac wing; inguinal, external, internal iliac lymph nodes. Upper thoracic spine (suspicious)
35	N/A	N/A	CT, bone scan	T9;T10;T11; right 1st rib; right AC joint; right 5th rib; L5; bilateral pelvis. iliac lymph nodes; sigmoid mesentery node
36	7	PT3b	CT, bone scan	Innumerable mets throughout all visualised bones, vertebrae

Note: On average, metastatic sites were recorded 2.2 ± 0.6 years prior to the onset of abiraterone or enzalutamide treatment.
Abbreviations: GS, Gleason score; CT, Computed tomography; FNA, fine needle aspiration; MRI, magnetic resonance imaging; DRE, digital rectal exam; SI, Sacroiliac; L2, L2 lumbar vertebrae; T3, Third thoracic vertebra; S1,S2, sacral spine; AC, acromioclavicular.

Tumor stage guide[2]

T2, PT2	The tumor is found only in the prostate, not other parts of the body.
PT2a	The tumor involves one-half of 1 side of the prostate.
PT2b	The tumor involves more than one-half of 1 side of the prostate but not both sides.
T2C, PT2c	The tumor has grown into both sides of the prostate.
T2N1	The cancer has spread to the regional (pelvic) lymph node(s).
T3, PT3	The tumor has grown through the prostate on 1 side and into the tissue just outside the prostate.
T3A, PT3a	The tumor has grown through the prostate either on 1 or both sides of the prostate.
T3b, PT3b	The tumor has grown into the seminal vesicle(s).
T3c	The tumor has invaded one or both of the seminal vesicles.

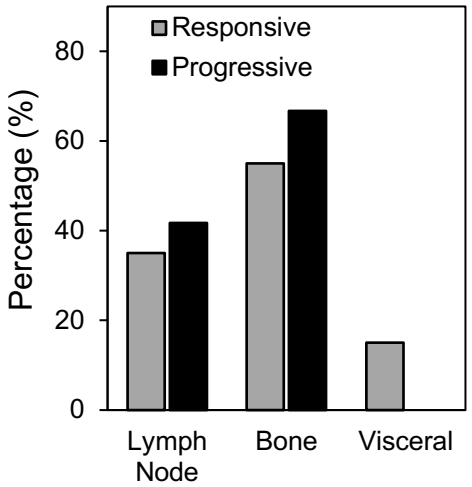


Figure S1. Percentage of mCRPC patients with lymph node, bone or visceral metastases. Patients were considered to have a lymph node metastatic incidence if they had a recording of at least one lymph node metastases (referring to Tables S2 and S3), and likewise for bone and visceral metastases. Percentage of lymph node metastases = # of lymph node metastases / total metastases. Progressive patients exhibit a higher incidence of bone metastases relative to responsive patients (refer to Materials and Methods for progressive vs. responsive definition).

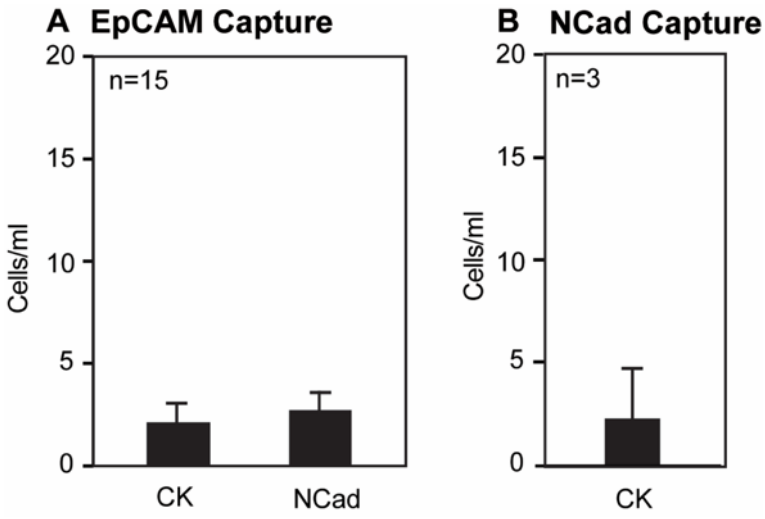


Figure S2. Healthy donor false positive cells captured in the microfluidic device. A) Target cells are captured with EpCAM-MNPs and identified as $DAPI^+/CK^+/CD45^-$ or $DAPI^+/NCad^+/CD45^-$. The false positive counts for EpCAM capture with the microfluidic device is 2 ± 1 Ck^+ cells/ml and 3 ± 1 N-cadherin$^+$ cells/ml. B) Cells are captured with N-cadherin- MNPs and identified as $DAPI^+/CK^+/CD45^-$. The false positive counts for N-cadherin capture with the microfluidic device is 3 ± 2 Ck^+ cells/ml.

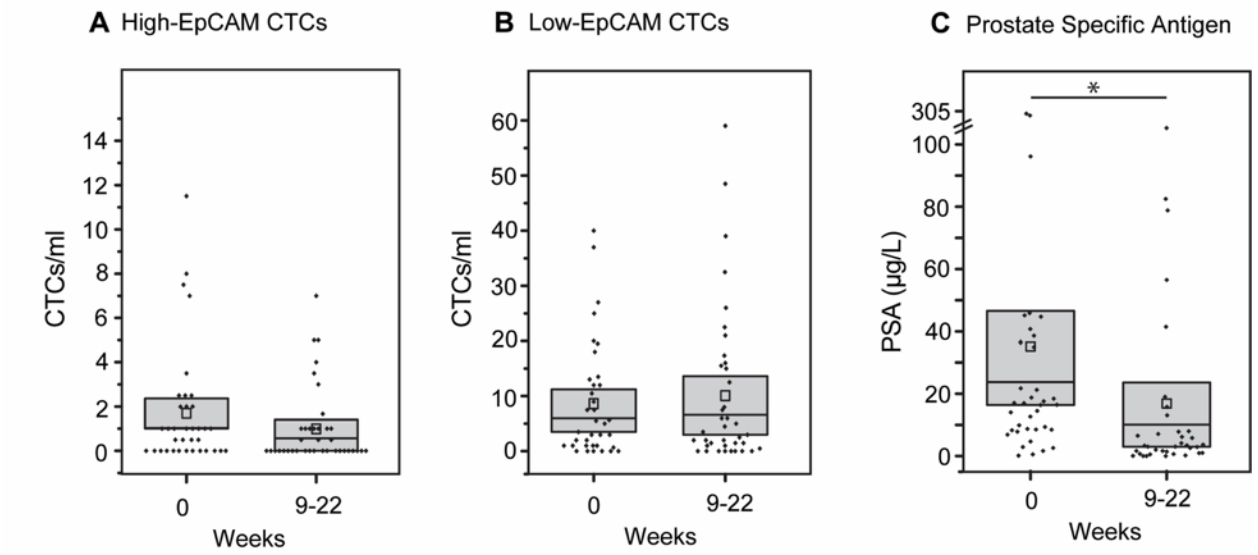


Figure S3. Cytokeratin CTC profiles and PSA levels for mCRPC patients. A)- B) Cytokeratin- positive CTC profiles for patients receiving enzalutamide or abiraterone. CTCs are separated based on High- EpCAM (zone 1 and zone 2) and Low- EpCAM (zone 3 and zone 4) populations. CTC profiles are shown for 0- and 9-22 weeks on- treatment. C) Prostate specific antigen (PSA) levels for patients receiving enzalutamide or abiraterone. Box plots represent standard error of the mean. Statistics were performed using Mann Whitney non-parametric analysis. *p<0.05. Each dot represents an individual patient CTC count or PSA value. CTCs are $DAPI^{+}/CK^{+}/CD45^{-}$.

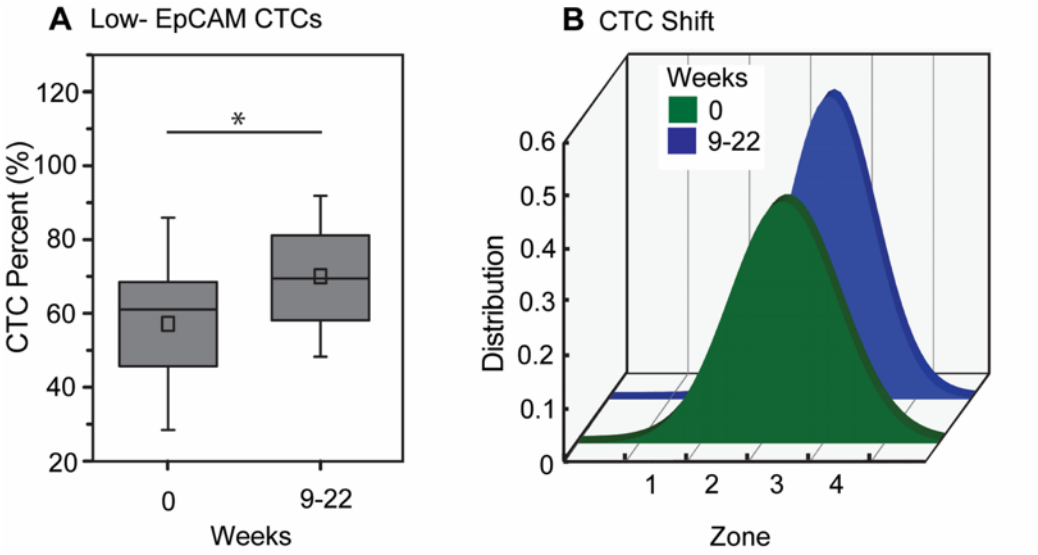


Figure S4. Phenotypic shift of CTCs towards low- EpCAM zones. A) Percentage of low- EpCAM (zone 4) CTCs relative to total CTCs. CTCs are $DAPI^{+}/CK^{+}/CD45^{-}$. Box plots represent 25th and 75th percentile. The mean is shown as the central square, with the median depicted as a line. Error bars represent the standard deviation. Statistics are performed with two-sample t-test. *p<0.05 is significant. B) Normal distribution of CTCs over the treatment period of 9-22 weeks. Median zone values for 0 and 9-22 weeks of treatment are 3.3 CTCs/ml and 3.5 CTCs/ml respectively.

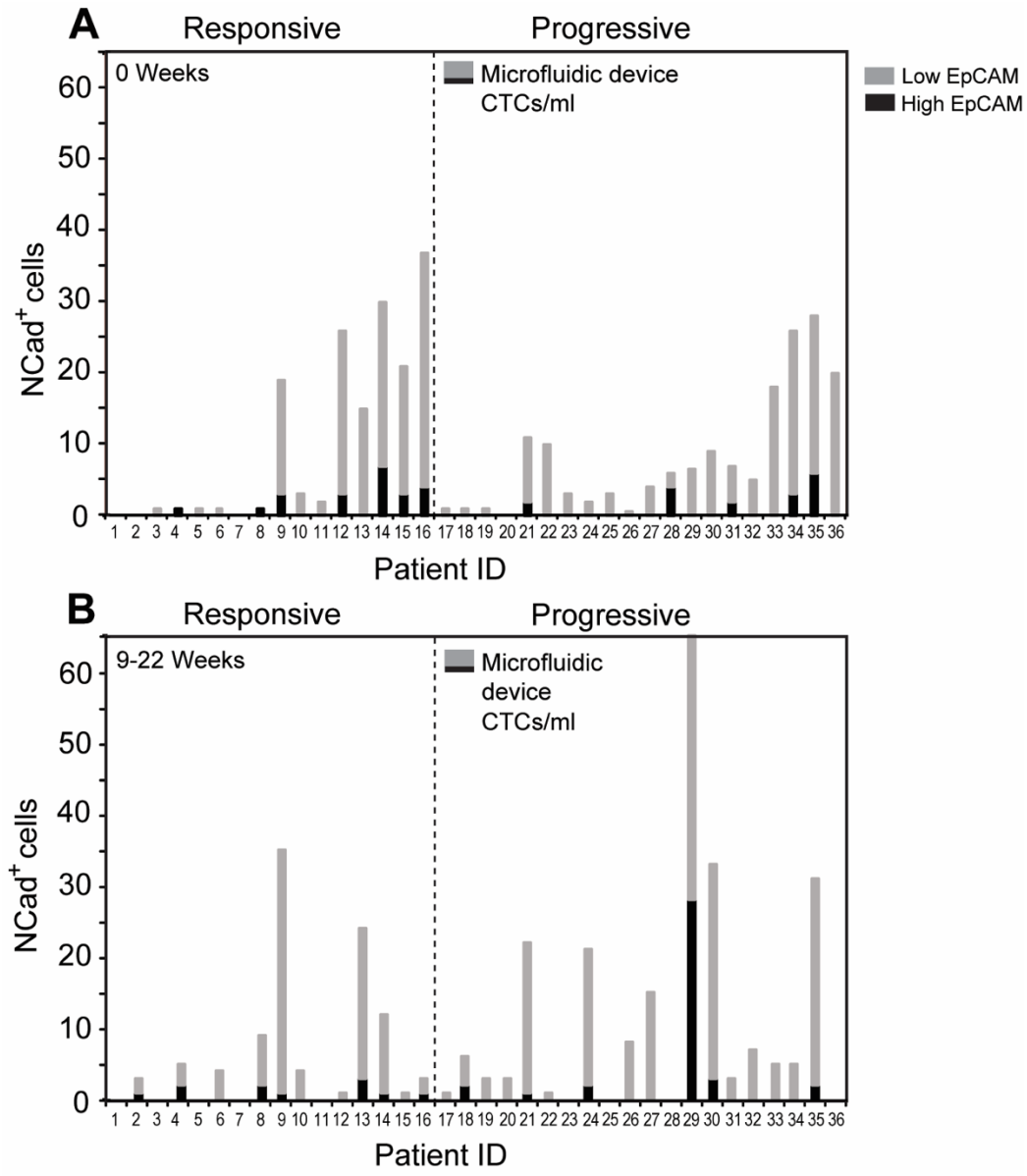


Figure S5. Temporal N-cadherin CTC profiles from prostate cancer patients. CTC counts were profiled with the microfluidic device (CTCs/ml) over A) 0 weeks, B) 9-22 weeks on- treatment. CTCs were captured using EpCAM magnetic nanoparticles and identified as $DAPI^{+}/NCad^{+}/CD45^{-}$. Microfluidic device CTC counts were divided into CTCs captured in low-EpCAM zones 1 and 2 (light grey) and high-EpCAM zones 3 and 4 (black). CTC profiles were obtained from 36 mCRPC patients receiving enzalutamide or abiraterone. No bar indicates 0 CTC count. The dotted line separates PSA responsive vs. progressive patients, as defined according to PCWG3 criteria.

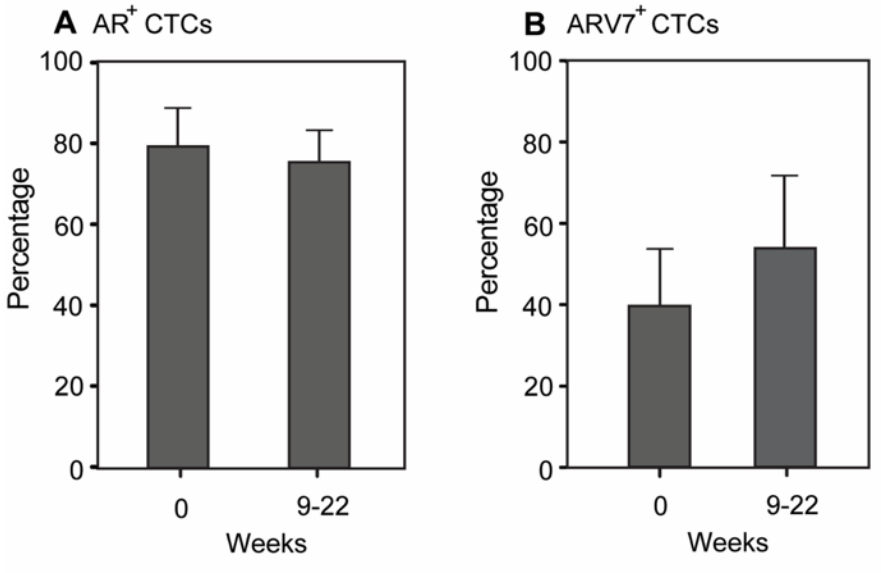


Figure S6. Androgen receptor biomarker expression levels for mCRPC CTCs. A) Percentage of androgen receptor (AR) CTCs: Cytokeratin CTCs from mCRPC patients. B) Percentage of androgen receptor variant 7 (ARV7) CTCs: Cytokeratin CTCs from mCRPC patients. CTC counts are obtained at 0- and 9-22-weeks on-treatment. CTCs were identified as $DAPI^+/CK^+/CD45^-$ or $DAPI^+/CK^+/AR^+/CD45^-$ or $DAPI^+/CK^+/ARV7^+/CD45^-$ and counts were obtained from separate devices. CTC data is obtained from 36 prostate cancer patients receiving abiraterone or enzalutamide. Zone 3 and zone 4 counts were considered for CTC ratio analysis. CTCs were captured with EpCAM- MNPs.

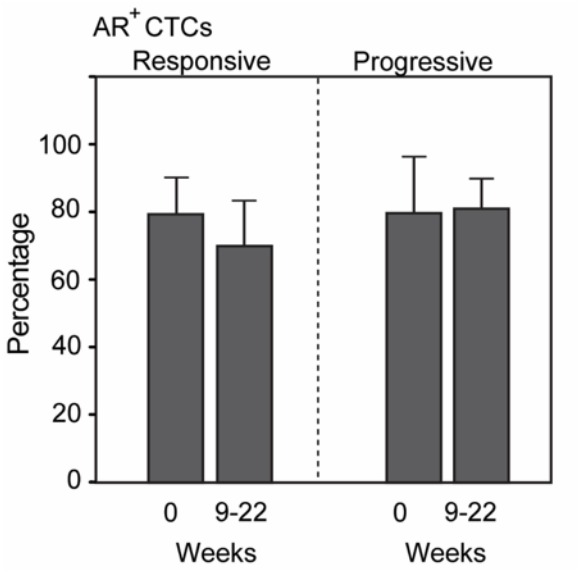


Figure S7. Androgen receptor biomarker expression levels for progressive and responsive mCRPC CTCs. Percentage of androgen receptor (AR) CTCs: Cytokeratin positive CTCs for progressive versus responsive patients (refer to Materials and Methods for progressive vs. responsive definition). Ratios were obtained from CTC counts at 0- and 9-22 weeks on- treatment. Zone 3 and zone 4 CTCs are considered for analysis. CTC data is obtained from 36 prostate cancer patients receiving abiraterone or enzalutamide. CTCs are identified as either $DAPI^+/CK^+/CD45^-$ or $DAPI^+/CK^+/AR^+/CD45^-$ and counts were obtained from separate devices. CTCs were captured with EpCAM- MNPs.

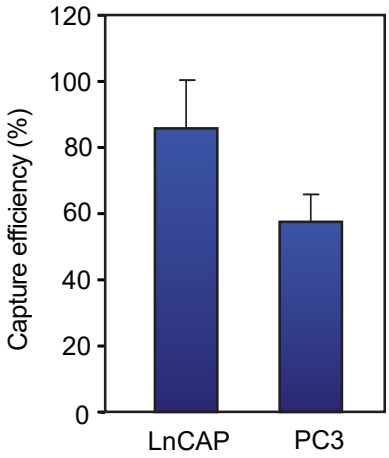


Figure S8. N-cadherin capture efficiency. LnCAP and PC3 cells are captured with N-cadherin- MNPs in the microfluidic device. 100 cells are loaded into the device. Experiment was performed in triplicate.

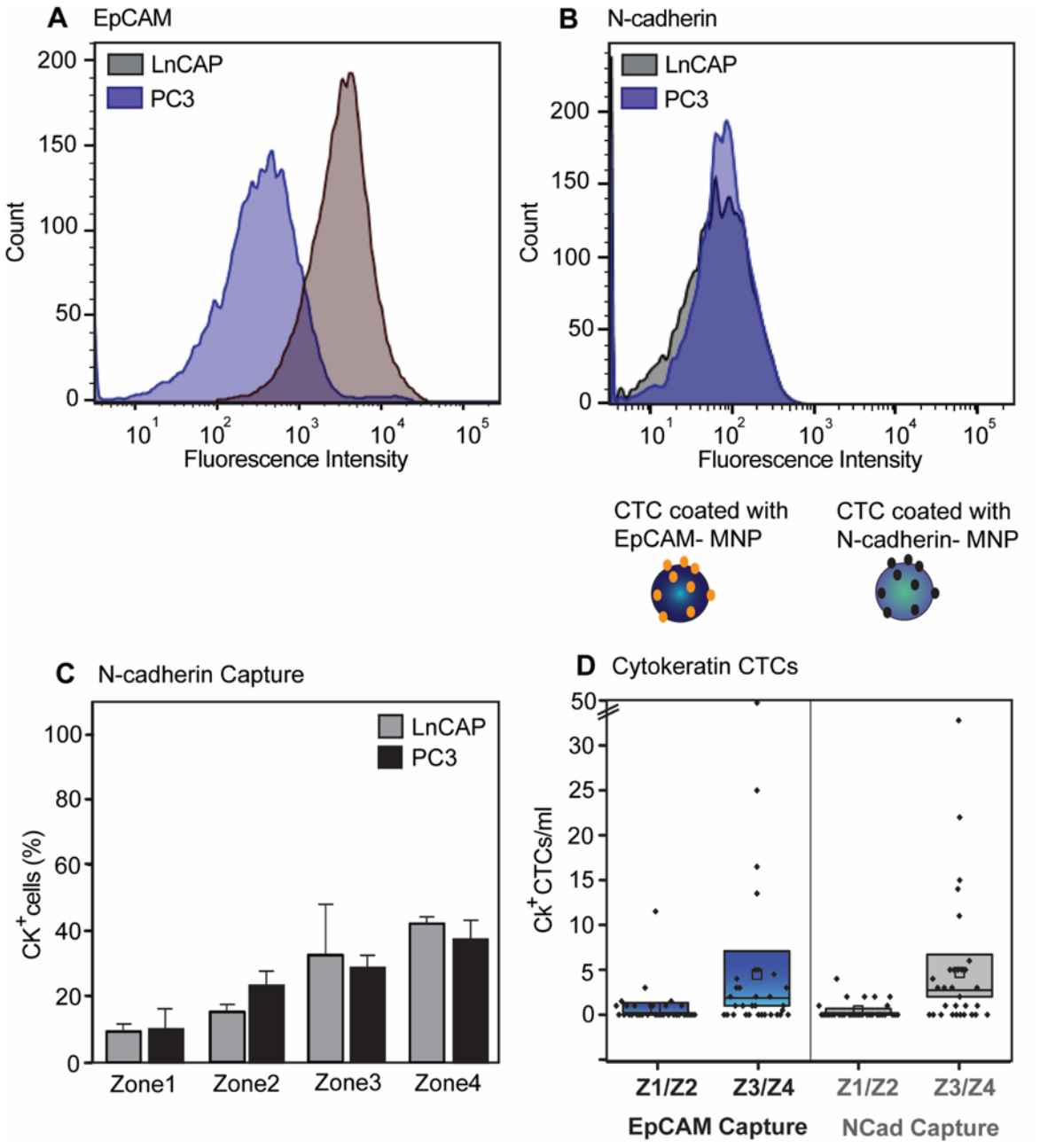
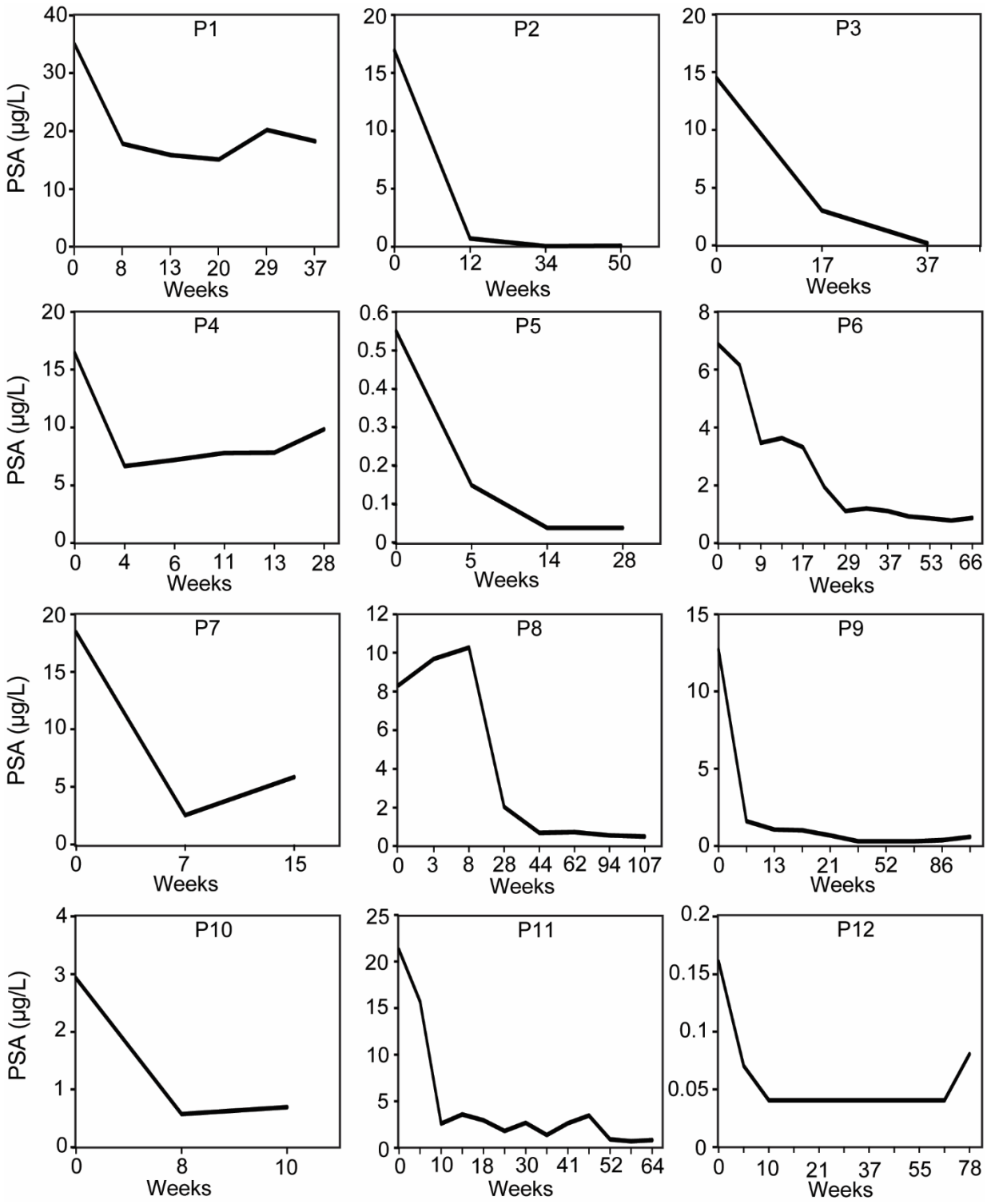


Figure S9. EpCAM- capture versus N-cadherin- capture of mCRPC CTCs. A,B) Flow cytometry analysis of EpCAM and N-cadherin in LnCAP and PC3 prostate cancer cells. 10,000 cells were analyzed per sample. C) LnCAP and PC3 cells profiled with the microfluidic device and captured with N-cadherin- MNPs. D) Cytokeratin positive CTCs profiled with EpCAM- MNPs and N-cadherin- MNPs. Patient received enzalutamide or abiraterone. CTCs are identified as $DAPI^+/CK^+/CD45^-$. CTC data is obtained from 36 prostate cancer patients.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Weeks
P2
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0 12 34 50
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P4
PSA (µg/L)
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5
0
0 4 6 11 13 28
Weeks
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0 5 14 28
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P6
8
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2
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0 9 17 29 37 53 66
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PSA (µg/L)
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PSA (µg/L)
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Weeks

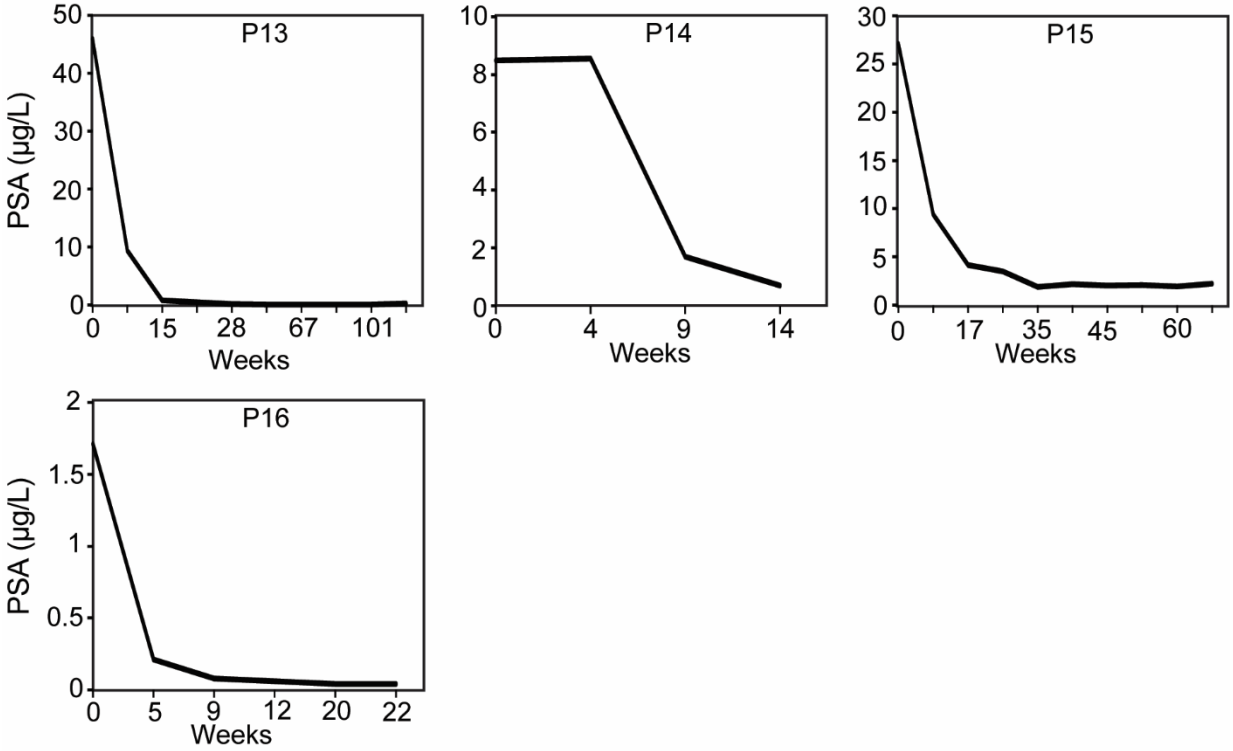
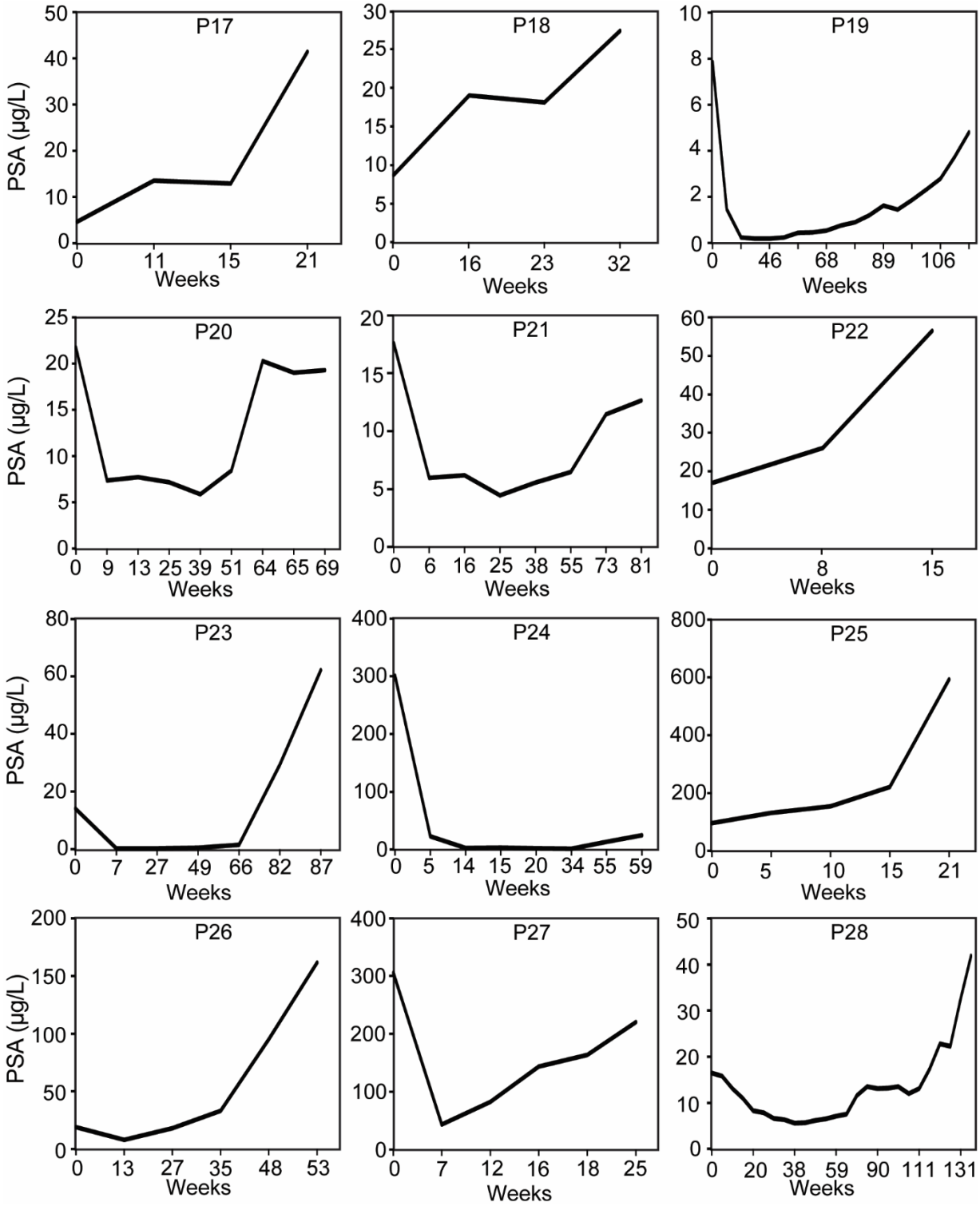


Figure S10. PSA profiles for responsive mCRPC patients receiving enzalutamide or abiraterone. PSA response is defined as a >50% decline from baseline measured twice 3 to 4 weeks apart.[1] The number of responsive patients is 16.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Weeks
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P19
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PSA (µg/L)
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Weeks

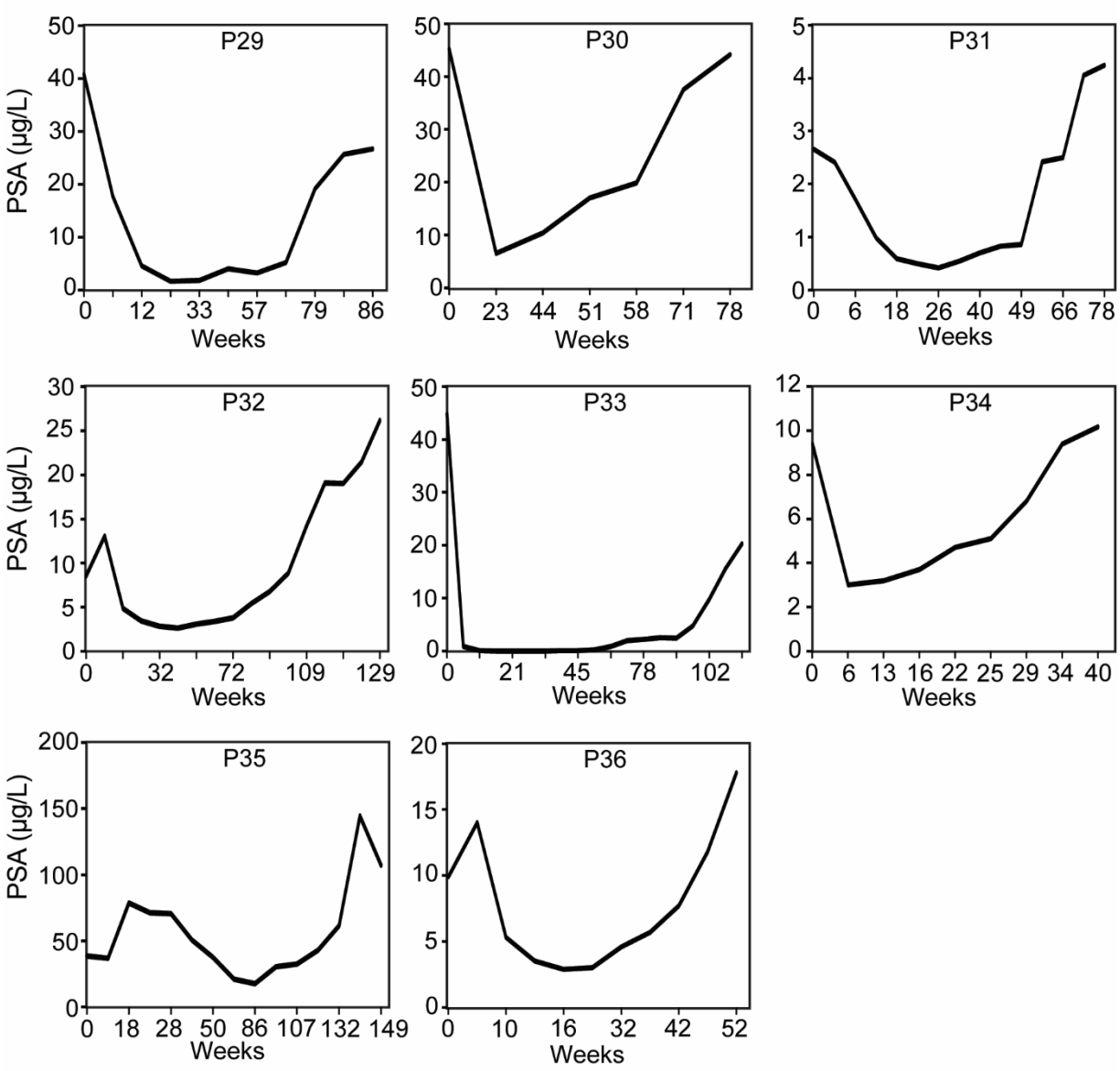

Figure S11. PSA profiles for progressive mCRPC patients receiving enzalutamide or abiraterone. PSA progression is defined as after a decline from baseline- record time from initiation of therapy to first PSA increase that is ≥ 25% and ≥ 2 ng/mL above the nadir, and which is confirmed by a second value 3 or more weeks later (ie, a confirmed rising trend). If there is no decline from baseline, PSA progression is defined as ≥ 25% and ≥ 2ng/mL after 12 weeks.[1] The number of progressive patients is 20.

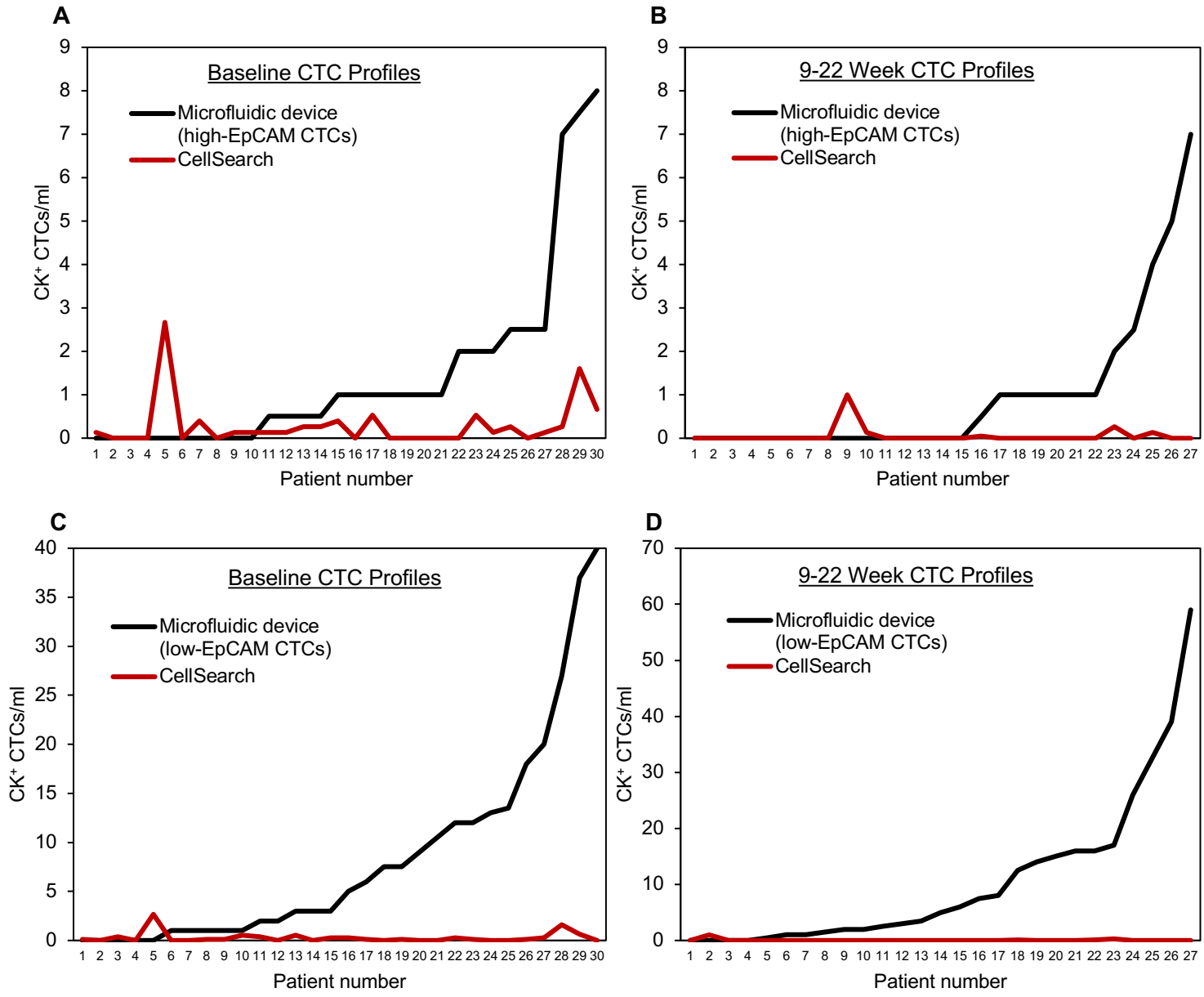

Figure S12. Microfluidic device and CellSearch CTC counts. A,B) High-EpCAM microfluidic device and CellSearch CTC counts for baseline and 9-22 week timepoints, respectively. C,D) Low-EpCAM microfluidic device and CellSearch CTC counts for baseline and 9-22 week timepoints, respectively. CTCs are $DAPI^{+}/Ck^{+}/CD45^{-}$ and reported as CTCs/ml. CTCs are organized from low-high counts with respect to the microfluidic device CTC counts. Patient numbers are assigned relative to this order for the purpose of visualizing an increasing trend.

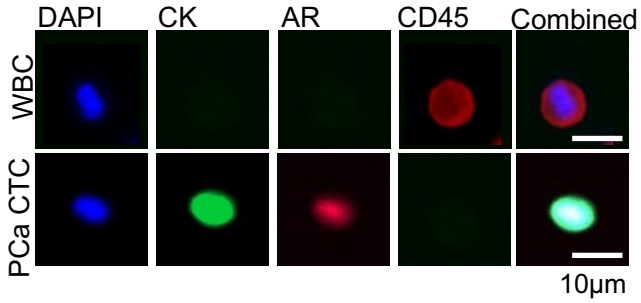

Figure S13. Immunostaining images of a white blood cell and a prostate cancer CTC. The CTC is identified as $DAPI^{+}/CK^{+}/AR^{+}/CD45^{-}$. The WBC is identified as $DAPI^{+}/CK^{-}/AR^{-}/CD45^{+}$.

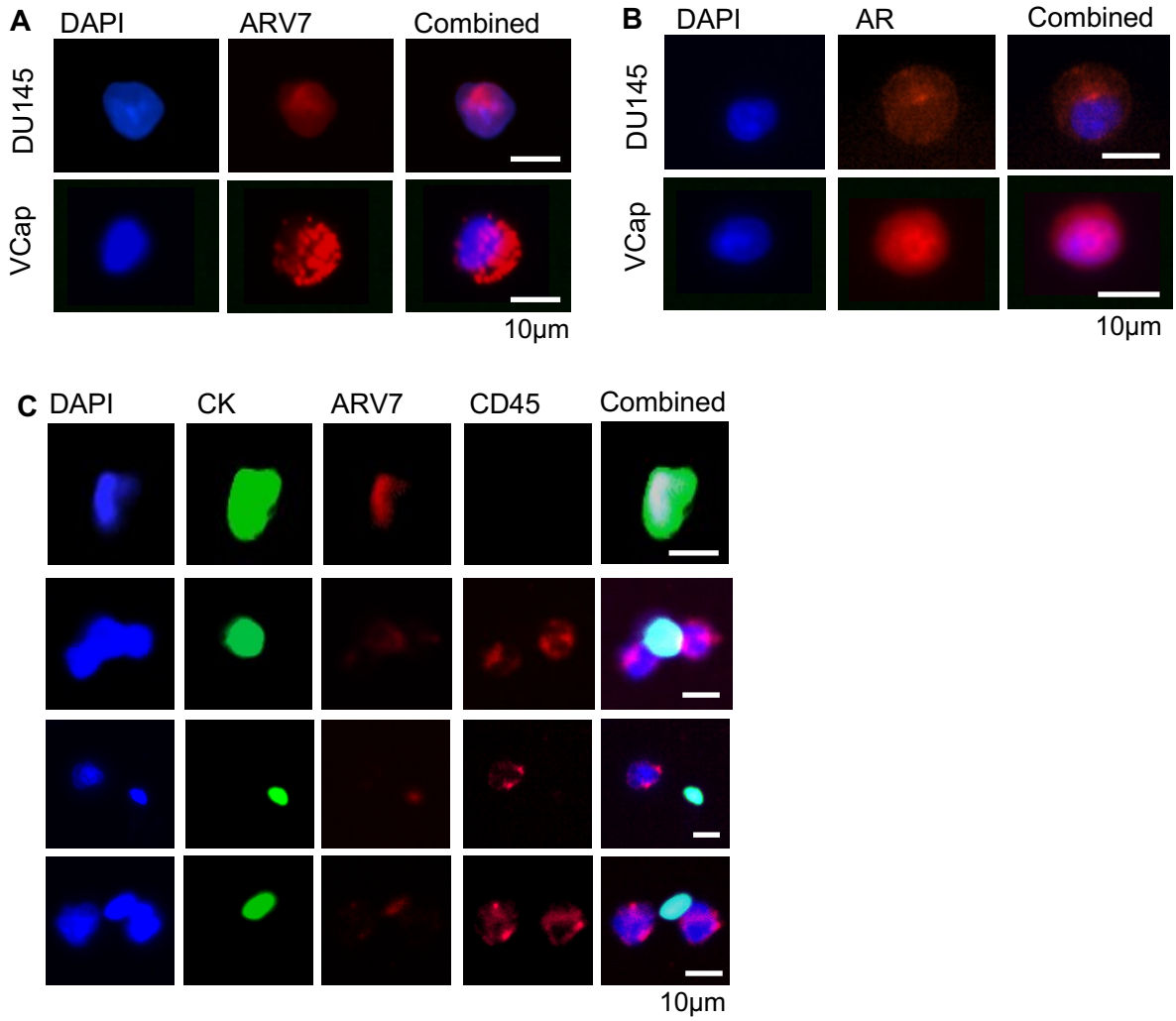

Figure S14. Androgen Receptor Variant 7 (ARV7) and AR immunostaining images of prostate cancer cells and WBCs. A,B) Immunostaining of DU145 and VCaP cells with antibodies against Androgen Receptor (AR) and Androgen Receptor Variant 7 (ARV7). C) Immunostained images of mCRPC patient CTCs. Cancer cells are $DAPI^{+}/CK^{+}/AR^{+}/CD45^{-}$ or $DAPI^{+}/CK^{+}/ARV7^{+}/CD45^{-}$ and WBCs are $DAPI^{+}/CK^{-}/ARV7^{-}/CD45^{+}$.

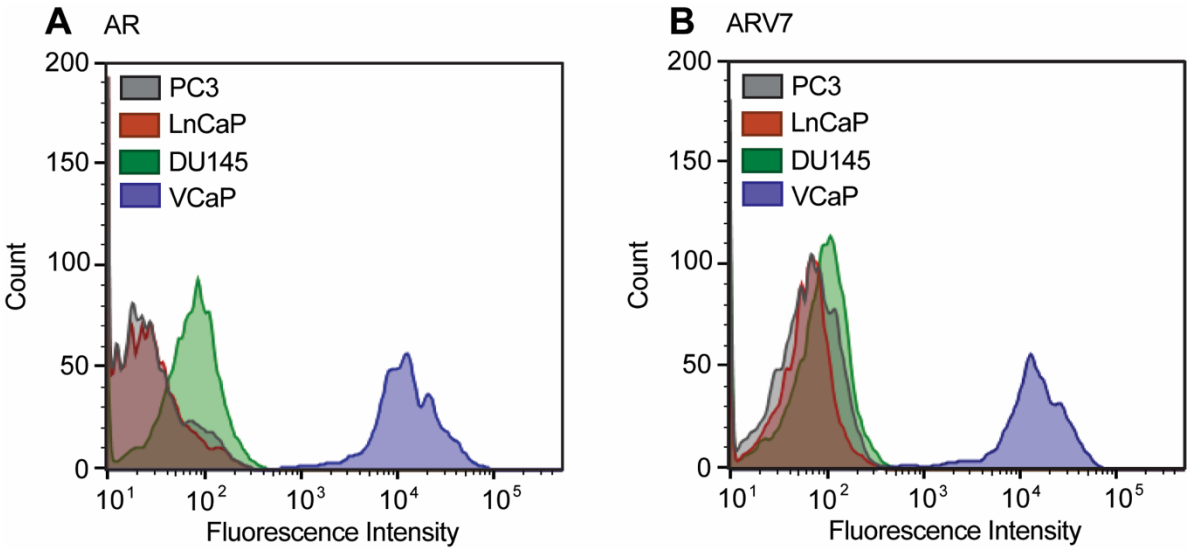

Figure S15. Androgen Receptor protein expression in prostate cancer cells. Flow cytometry analysis of A) AR and B) ARV7 in PC3, LnCaP, DU145 and VCaP cells. 3000 cells were analyzed per sample. Low-AR expressing cells include DU145, PC3 and LnCaP, while high-AR expressing cells include VCaP.[3,4]

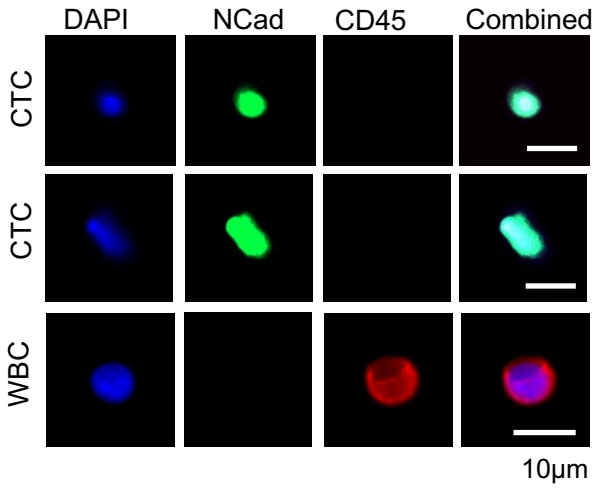

Figure S16. N-cadherin immunostaining images of prostate cancer CTCs and a WBC. CTCs are DAPI$^+$/NCad$^+$/CD45$^-$ and WBCs are DAPI$^+$/NCad$^-$/CD45$^+$.

References

(1) Scher, H. I.; Morris, M. J.; Stadler, W. M.; Higano, C.; Basch, E.; Fizazi, K.; Antonarakis, E. S.; Beer, T. M.; Carducci, M. A.; Chi, K. N.; Corn, P. G.; de Bono, J. S.; Dreicer, R.; George, D. J.; Heath, E. I.; Hussain, M.; Kelly, W. K.; Liu, G.; Logothetis, C.; Nanus, D., et al. Trial Design and Objectives for Castration-Resistant Prostate Cancer: Updated Recommendations From the Prostate Cancer Clinical Trials Working Group 3. *J. Clin. Oncol.* **2016**, *34* (12), 1402-1418.

(2) Cheng, L.; Montironi, R.; Bostwick, D. G.; Lopez-Beltran, A.; Berney, D. M. Staging of prostate cancer. *Histopathology* **2012**, *60* (1), 87-117.

(3) Alimirah, F.; Chen, J.; Basrawala, Z.; Xin, H.; Choubey, D. DU-145 and PC-3 human prostate cancer cell lines express androgen receptor: implications for the androgen receptor functions and regulation. *FEBS Lett.* **2006**, *580* (9), 2294-300.

(4) Liu, L.L; Xie, N.; Sun, S.; Plymate, S.; Mostaghel, E.; Dong X. Mechanisms of the androgen receptor splicing in prostate cancer cells. *Oncogene* **2014**, *33* (24), 3140–3150.