Supplemental Materials and Methods

Preparation of Paramagnetic Particles (PMPs)

An antibody to the epithelial cell adhesion molecule (EpCAM, R&D Systems, USA) was biotinylated according to the Dynabeads® FlowComp™ Flexi manufacturer's instructions (Life Technologies, USA). Each batch of antibody was tested for capture efficiency and shown to deplete at least 85% of LNCaPs from the capture well of a VERSA.

Streptavidin coupled PMPs from the Dynabeads® FlowComp™ Flexi kit (Life Technologies, USA) were used at a concentration of 250 µg per reaction for all experiments. The PMPs were washed twice and resuspended in 0.1% Tween-20 in PBS prior to addition of biotinylated anti-EpCAM. The PMPs and antibodies were mixed for 30 minutes at RT followed by three washes and resuspension in 25 µL of 0.1 % BSA in PBS.

Patient Sample Processing

Blood was collected in Cellsave tubes (Jansen Diagnostics) for AR localization (7.5 mL) and K2EDTA tube (BD Biosciences) for nucleic acid isolation (15 mL). Blood was diluted with equal volume of HBSS (Lonza) and underlayed with 10 mL of Ficoll-Paque Plus (GE, USA). Gradients were spun at 980xg for 40 minutes and the" buffycoat" layer containing mononuclear cells was transferred to a new tube and washed with CTC binding buffer (1x phosphate buffered saline (PBS) with 0.1% BSA + 2 mM EDTA). For AR nuclear localization studies, mononuclear cells were fixed with BD Cytofix™ (BD Biosciences) for 30 minutes on ice and washed once in CTC binding buffer and resuspended in CTC binding buffer. For nucleic acid extraction, cells were CD45 depleted using CD45 Microbeads (Miltenyi Biotec) following manufacturer's instructions.

VERSA operation

Live Cell VERSA

EpCAM conjugated PMPs (25 µL), 20 µL 0.1 % Tween 20 in PBS and live CD45-depleted PBMCs suspended in CTC binding buffer were added to the input well. Volume was increased with CTC binding buffer to fill capture well. Silicone oil was added to the trapezoid between aqueous solutions. The device containing the sample was rotated for 30 min at 4 °C.The PMP bound cells were then moved through the first oil trapezoid using a handheld magnet (B333-N52, K&J Magnetics, USA) and transferred into the extracellular staining well where it was mixed by gently pipetting. Cells were stained for 30 minutes at 4 °C with Hoechst 33342 (Life Technologies), Anti-CD45, Anti CD34 (Biolegend) and Anti-Epcam (Abcam). The bead-bound cells were then transferred to the release well containing release buffer (Life Technologies), dispersed by pipetting in the front well and the magnet was used to pull the unbound PMPs through the membrane to the back sieve well where they were collected by removing release buffer. The sieve well was then filled with fresh release buffer and incubated 10-30 minutes to release cells. After incubation, PMPs were again pulled through to the back sieve well and removed.

Nucleic Acid Extraction

Nuclease free water (15 µL) was added to both mRNA and DNA extraction wells. Fluid was removed gently from the back sieve well using a pipette until approximately 10 µL of sample was left in the front well. Olive oil (Unilever, USA) was added to the back sieve well. The olive oil pinned within the back sieve well creates a plug which prevents nucleic acids from

escaping the front well following cell lysis. mRNA lysis/binding buffer (15 µL) (10 mM Tris-HCl, 500 mM lithium chloride , 1 % Igepal® CA-630 (Sigma-Aldrich, USA), 5 mM ethylenediaminetetraacetic acid (EDTA), 5 mM dithiothreitol, pH 7.5) containing 30 µg oligo(dT) Dynabeads® (Life Technologies, USA) was added to the front sieve well. To complete filling of the device, 60 µL silicon oil (Fisher, USA) was added to the well connecting sieve and nucleic extraction wells. After 5 minutes, a magnet was used to pull olgio(dt) PMPs across the front until the PMPs reached the mRNA output well. Next, 25 µL of DNA binding buffer (10 mM Tris-HCL, 6 M GTC, 0.1 % Igepal® CA-630, pH 7.5) containing 1 µL MagneSil® PMPs (Promega, Madison) was added to the front sieve well. After 5 minutes, the MagneSil® PMPs were transferred to the DNA output well using the permanent magnet.

Fixed Cell VERSA

CTCs are bound to EpCAM labeled PMPs as described above for the live cell VERSA. The PMP bound cells are then moved through the first oil trapezoid using a handheld magnet and transferred into the extracellular staining well containing anti-CD45 antibody conjugated to PE (Biolegend, USA) and Hoechst. The samples were incubated for 30 minutes at 4°C. The PMP bound cells were then transferred to the sieve well containing release buffer (Invitrogen, USA). The cells were dispersed by pipetting in the front well, and the magnet was then used to pull the unbound PMPs through the membrane to the back sieve well where the PMPs were collected by removing release buffer. The sieve well was then filled with fresh release buffer and incubated 10-30 minutes to release cells. After incubation, PMPs were again pulled through to the back sieve well and removed. Cells were permeabilized in the sieve well for 30 minutes by the addition of 1 % Tween 20 + 0.05% Saponin in PBS. This was followed by blocking for 30 min at

room temperature with 3% BSA +0.1% Tween in PBS. Cells were then incubated overnight at 4 °C with anti-Pan-cytokeratin (C-11) and anti-androgen receptor antibody (Cell signaling, USA). The samples were washed 3 times with 0.1 % BSA in PBS. A Donkey anti-Rabbit secondary antibody labeled with AlexaFluor-488 was added for 1 hour at RT followed by 5 washes with 0.1 % BSA in PBS. Samples were imaged within device and collected using Nikon Eclipse Ti and NIS-Elements AR Microscope Imaging Software (Nikon, USA).

Supplementary Figures:

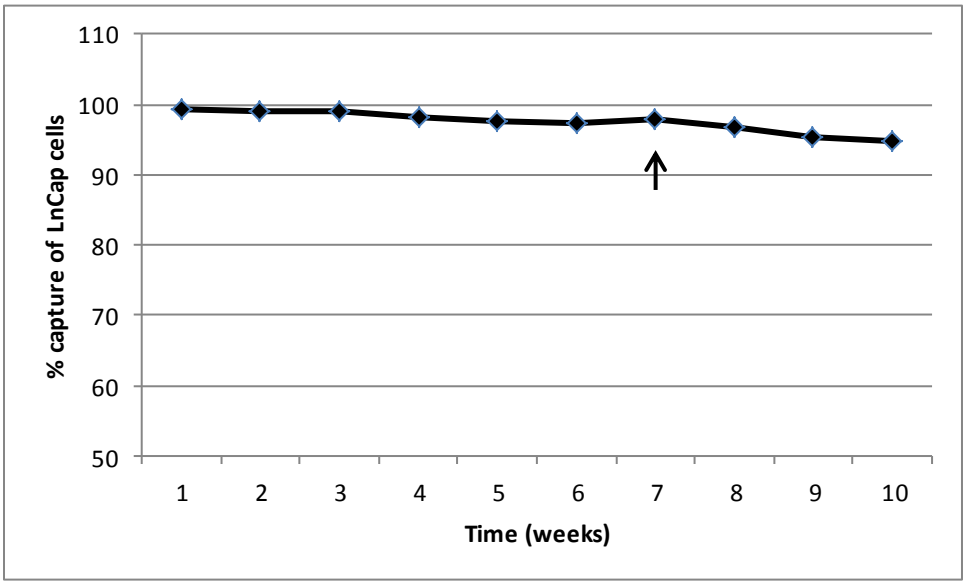

Figure S1. Longitudinal analysis of capture efficiency of Epcam antibody. Capture of LnCap cells from the input well of the VERSA was tested weekly for 10 weeks. Arrow indicates when a new aliquot of the antibody was aliquotted and used.

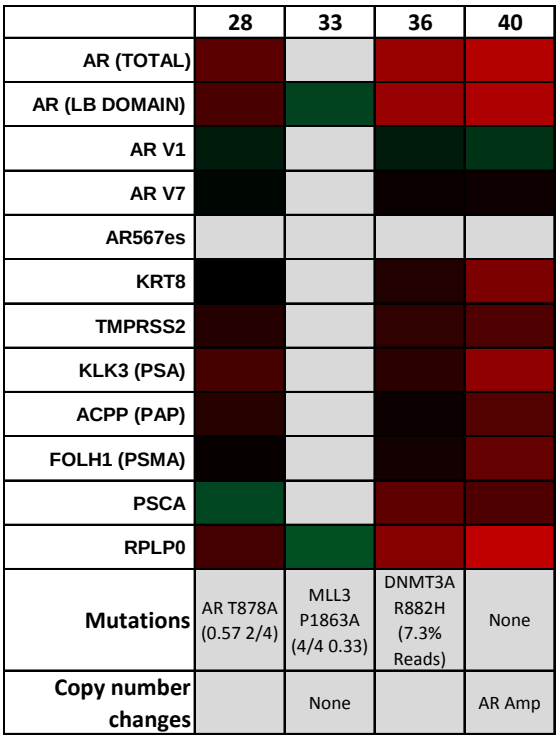

Figure S2. Multi-parametric analysis of gene expression, genomic profiling and AR protein analysis from captured CTCs. Rows show data from gene expression analysis (heatmap, as in Figure 3) and genomic sequencing (coverage, short variants with observed mutant allele frequencies, and copy number alterations detected) performed on select patients (listed at top).

Table S1. Catalog numbers of primers used for quantitative qPCR

Gene	Catalogue #
AR Exon 1-2 junction	Hs00907242_m1
AR Exon 4-5 junction	Hs00171172_m1
AR Splice variant V1	HuAR_V1
AR Splice variant V7	Hs04260217_m1
AR Splice variant V567es	Hs04260216_m1
KRT8	Hs01595539_g1
TMPRSS2	Hs01120965_m1
KLK3 (PSA)	Hs002576345_m1
ACPP (PAP)	Hs00173475_m1
FOLH1 (PSMA)	Hs00379515_m1
PSCA	Hs04177224_m1
RPLP0	4333761F

Table S2. Patients 1-17, AR protein intensity and localization

Patient	Age	Gleason Score	PSA	Current treatment	CTCs (#)
Prior to Beginning AR Targeting Therapy					
1	81	2+3	68.2	None	14
2	54	4+5	10.9	None	6
3	81	4+5	508	None	2
4	49	4+3	73.8	None	5
Responding to AR Targetting Therapy					
5	69	4+5	103	Abiraterone	4
6	63	NA	15.6	Abiraterone	2
7	73	3+3	1.98	ARN-509	2
8	72	4+5	2.94	ARN-509	4
9	61	4+4	6.86	Enzalutamide	3
10	59	4+5	10.9	Enzalutamide	3
11	58	3+5	25.6	Enzalutamide	2
Responding to Docetaxel					
12	73	4+5	92.4	Docetaxel	2
13	71	4+4	10.8	Docetaxel	8
14	69	4+4	68.7	Docetaxel	2
Disease Progression after AR Targeting Therapy					
15	81	4+5	246	Enzalutamide	14
16	64	4+3	131	Enzalutamide	120
17	55	4+5	228	Abiraterone	11

Table S3. Patient characteristics-gene expression studies

						Metastasis		Prior treatments				
	Age (yrs)	Gleason Score	PSA (at Blood draw)	Current treatment	Time since diagnosis (years)	Bone/ Lymph node	Viceral	Abiraterone	Enzalutamide	TAK700	Sipuleucel-T	Chemotherapy
18	55	4+5	377	Lupron and Enzalutamide	7	X	X					X
19	69	3+4	186	Abiraterone acetate	8	X	X		X			X
20	61	5+4	276.1	VT-464	5	X			X			X
21	65	3+3	760	Mitoxantrone	10	X			X	X		X
22	63	4+4	780	Enzalutamide	9	X				X		
23	77	Poorly differentiated	115	Abiraterone acetate	2	X						
24	78	3+4	45.2	None	1	X						
25	72	4+5	59.9	Abiraterone acetate	9	X						
26	68	4+5	115	Observation	17	X						
27	75	Poorly differentiated	291	Radium 223	3	X		X				X
28	65	5+5	16.3	Enzalutamide	2	X					X	X
29	66	Poorly differentiated	369.1	VT-464	12	X		X				
30	71	3+4	37.4	Abiraterone acetate	11	X						
31	75	5+4	0.76	Lupron, casodex, docetaxel	1	X	X					
32	69	3+4	0.32	TAK700	13	X						X
33	65	3+4	38.1	Enzalutamide	5	X					X	
34	69	4+5	13.3	Radium 223	7	X				X		
35	68	3+4	3.92	Lupron, casodex, docetaxel	11	X	X					
36	74	4+5	4.09	ARN-509	9							
37	73	4+5	12.3	TAK700	11	X						
38	67	4+3	12.4	TAK700	11	X						X
39	60	5+4	8.1	Abiraterone acetate	5	X						
40	62	4+5	0.07	Lupron, casodex, docetaxel	1	X						
41	83	3+3	7.56	Lupron and azacytidine	13	X						
42	65	4+5	28.1	Palliative radiation	3	X	X					X
43	61	3+5	17.6	Observation	13	X				X		X