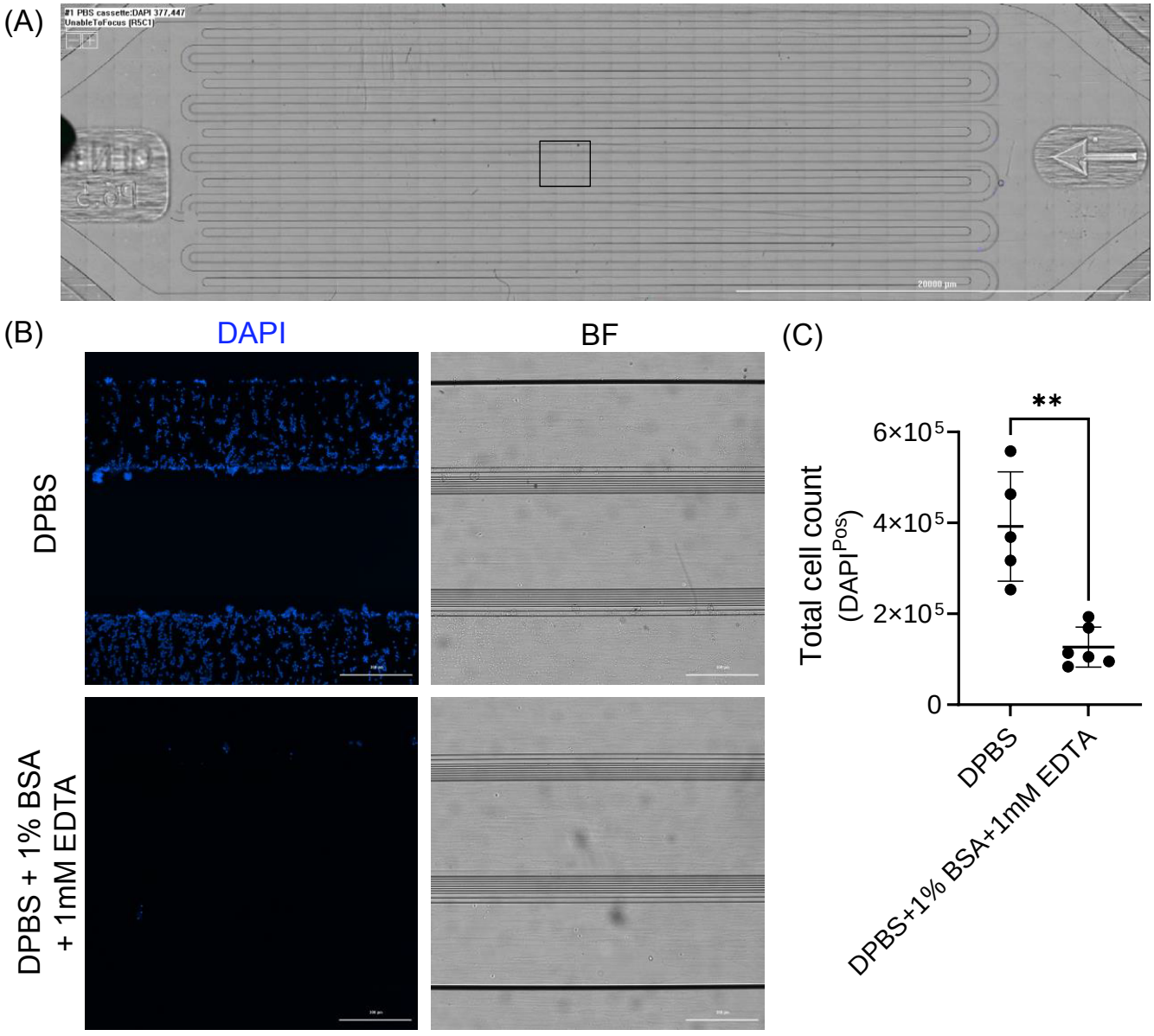
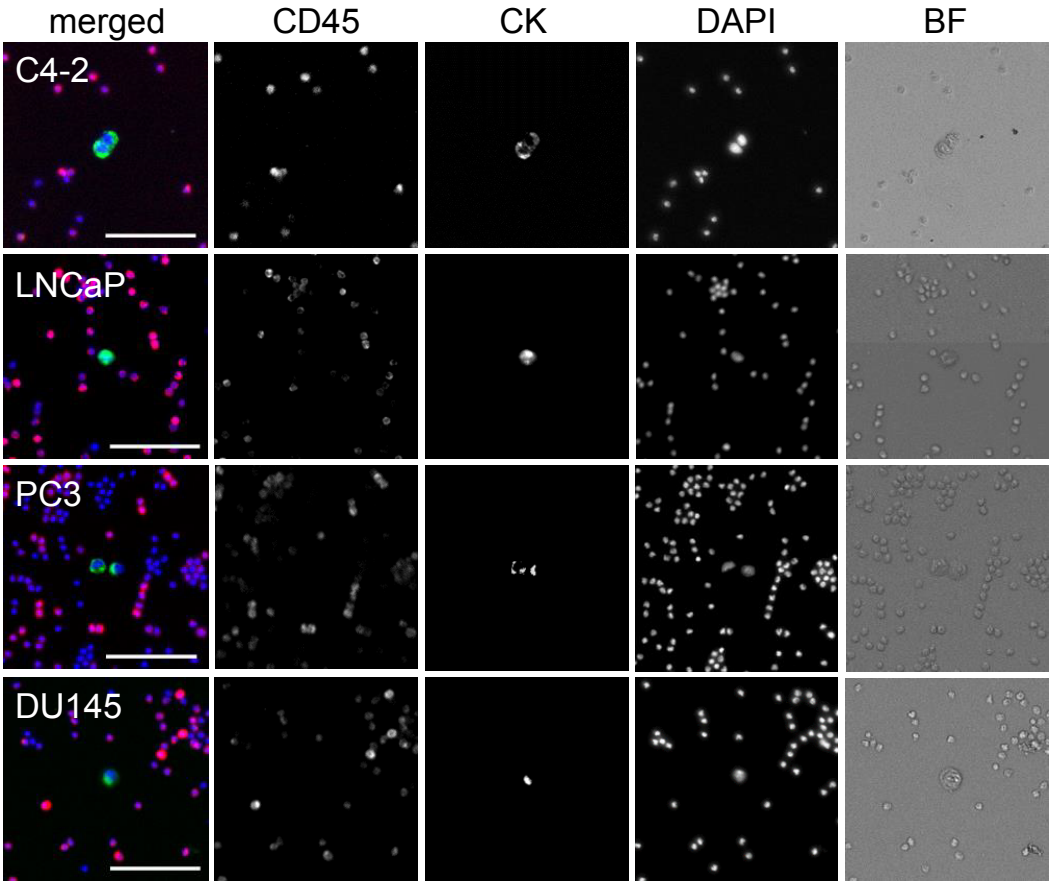


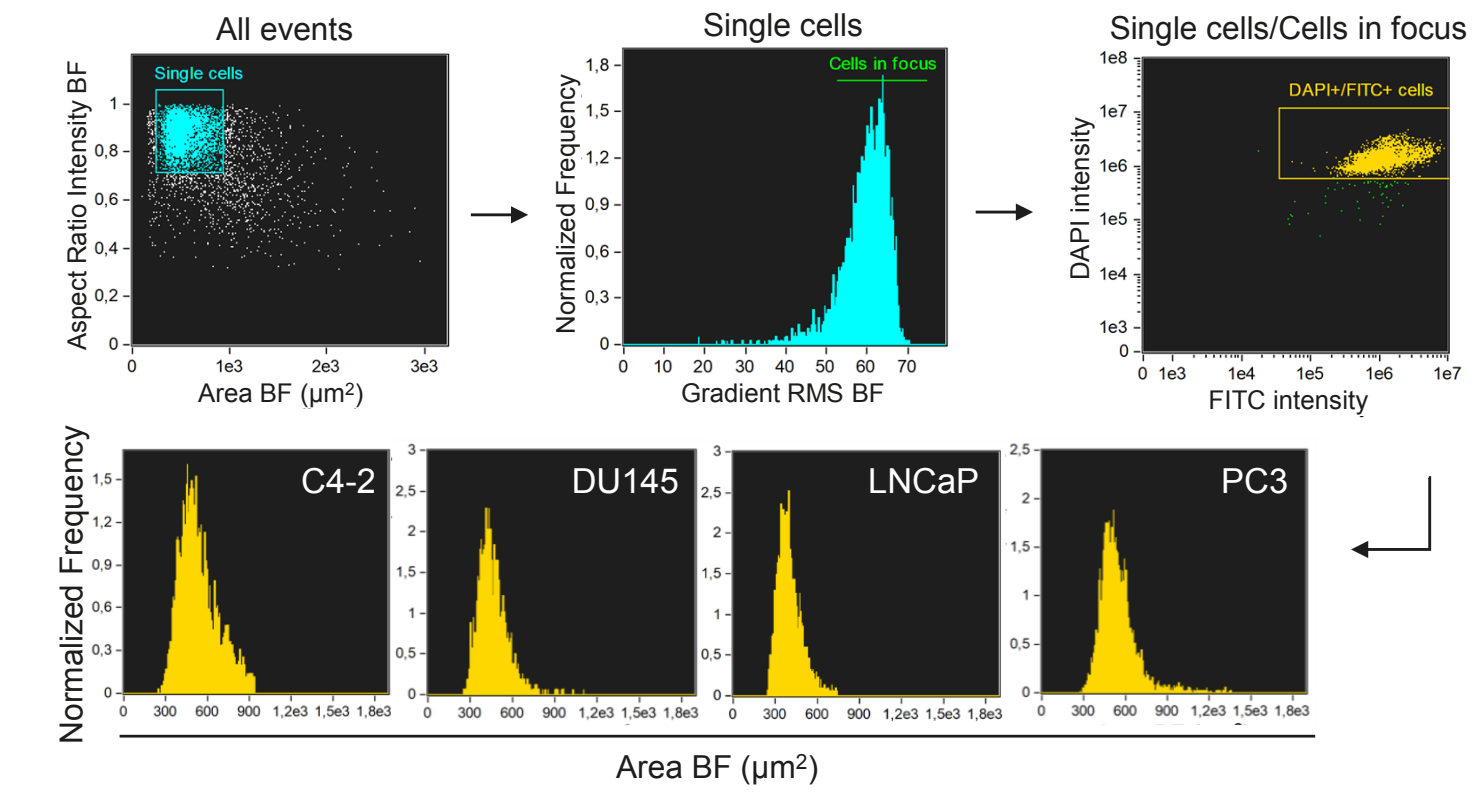
Supplementary Figure 1: Buffer optimization

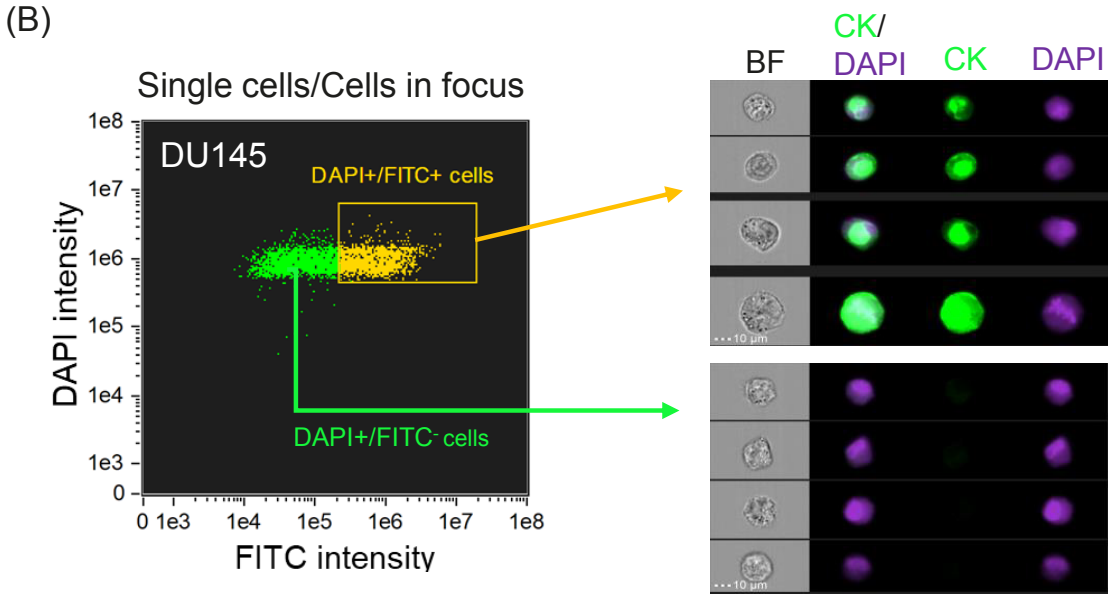
(A) Representative overview of the whole cassette after scanning. Scale bar is 2000 µm. The area in the black square is magnified to show representative images of the $DAPI^{Pos}$ cells retained in the cassette after cell harvest (B) Two different separation buffers were tested: DPBS or DPBS + 2% BSA. DAPI (blue) and BF (Brightfield). Scale bar is 300 µm (C) Total cell count in the cassette from four independent experiments after harvest. The $DAPI^{Pos}$ cells were automatically counted using the Gen5 version 3.10 software (threshold value = 5000, min. object size = 5µm, max. object size = 100µm). Data is presented as mean ± sd. Statistical analysis was performed using a non-parametric Mann-Whitney *U* test (p = 0.0043)



Supplementary Figure 2. Prostate cancer cells spiked into healthy donor blood

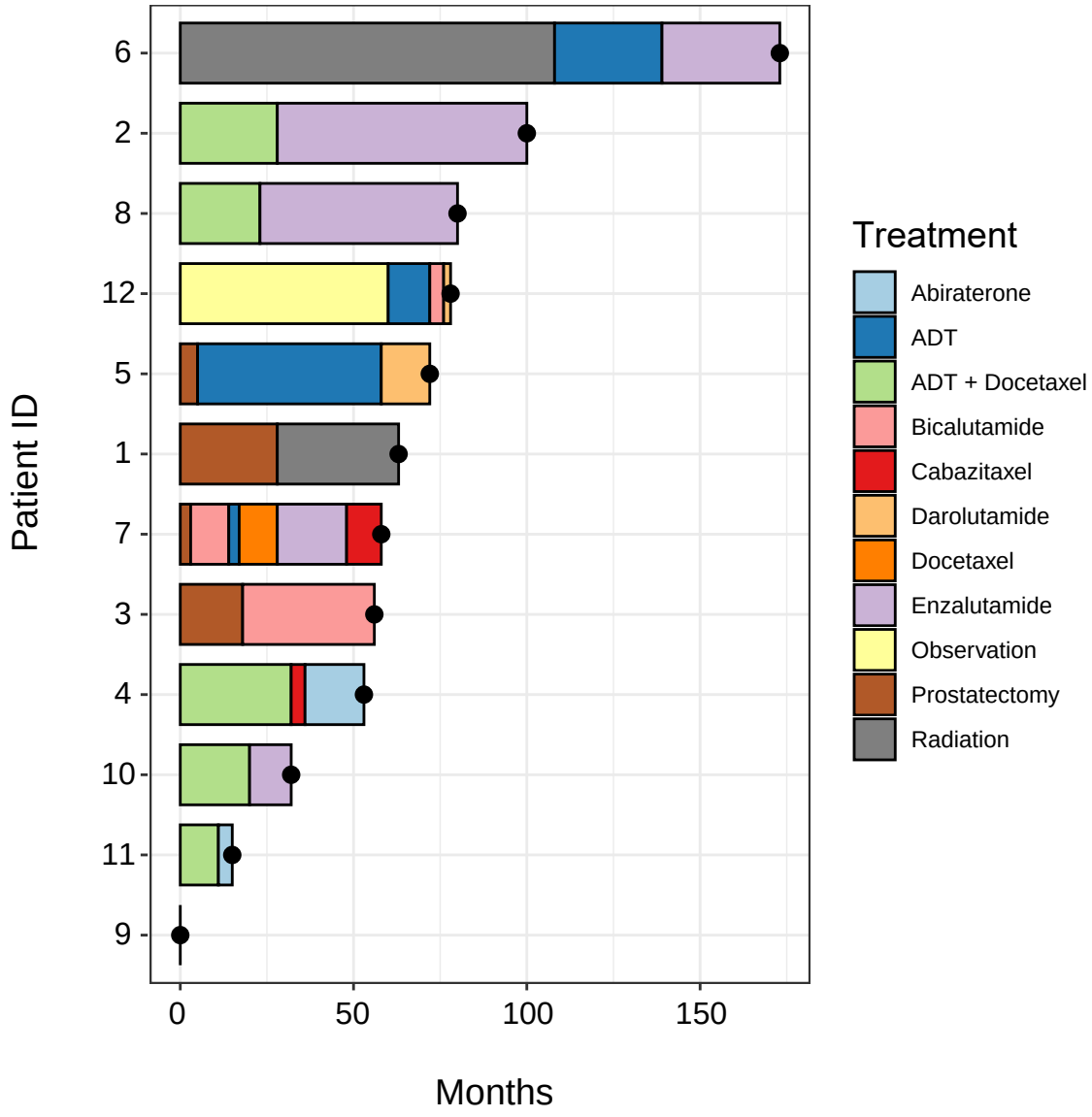
Representative images of the four prostate cell lines C4-2, LNCaP, PC3 and DU145 after being spiked into healthy donor blood following enrichment and in-cassette staining on the Parsortix® (ANGLE). The cells were stained with a cocktail of antibodies: anti-CD45 conjugated to APC (purple), anti-CK conjugated to FITC (green) and DAPI staining of the cell nucleus (blue). The samples were analysed on the CellCelector™ (Sartorius) and cells positive for CK and DAPI was considered CTCs. Scale bar is 100 µm.





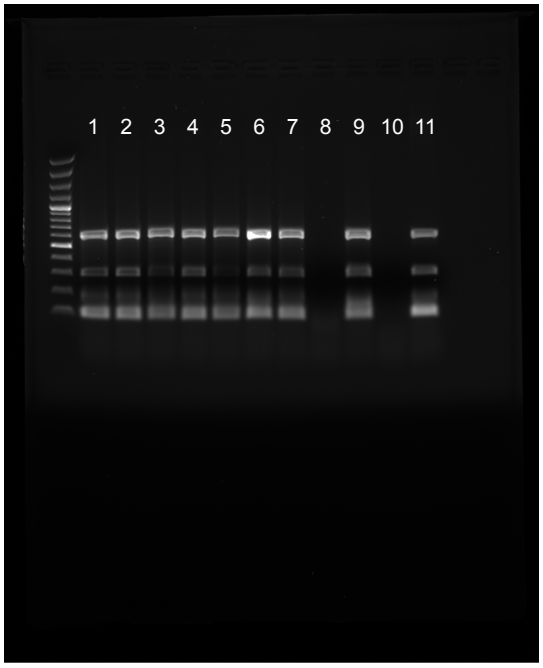
Supplementary Figure 3. Phenotypic characterization of prostate cancer cell lines

(A) The four prostate cell lines (PC3, DU145, C4-2 and LNCaP) were stained with antibodies targeting CK fluorescently conjugated to FITC and DAPI (cell nucleus) and analysed on the ImageStream flow cytometer. Single cells were gated based on the area (brightfield, BF) and the aspect ratio. The cells in focus were gated based on the Gradient RMS that measures the pixel contrast. The cells were then selected based on dual expression of CK and DAPI (B) Scatter plot of DAPI intensity vs. CK intensity of the DU145 cells.



Supplementary Figure 4. Treatment overview of metastatic prostate cancer patient cohort
The different colors represent the line of treatment given to each of the 12 patients in a period of 0 - 14.5 years. The black dot illustrates time of blood sample collection for CTC analysis. Abbreviations: ADT: Androgen deprivation therapy, PSMA: Prostate-specific membrane antigen

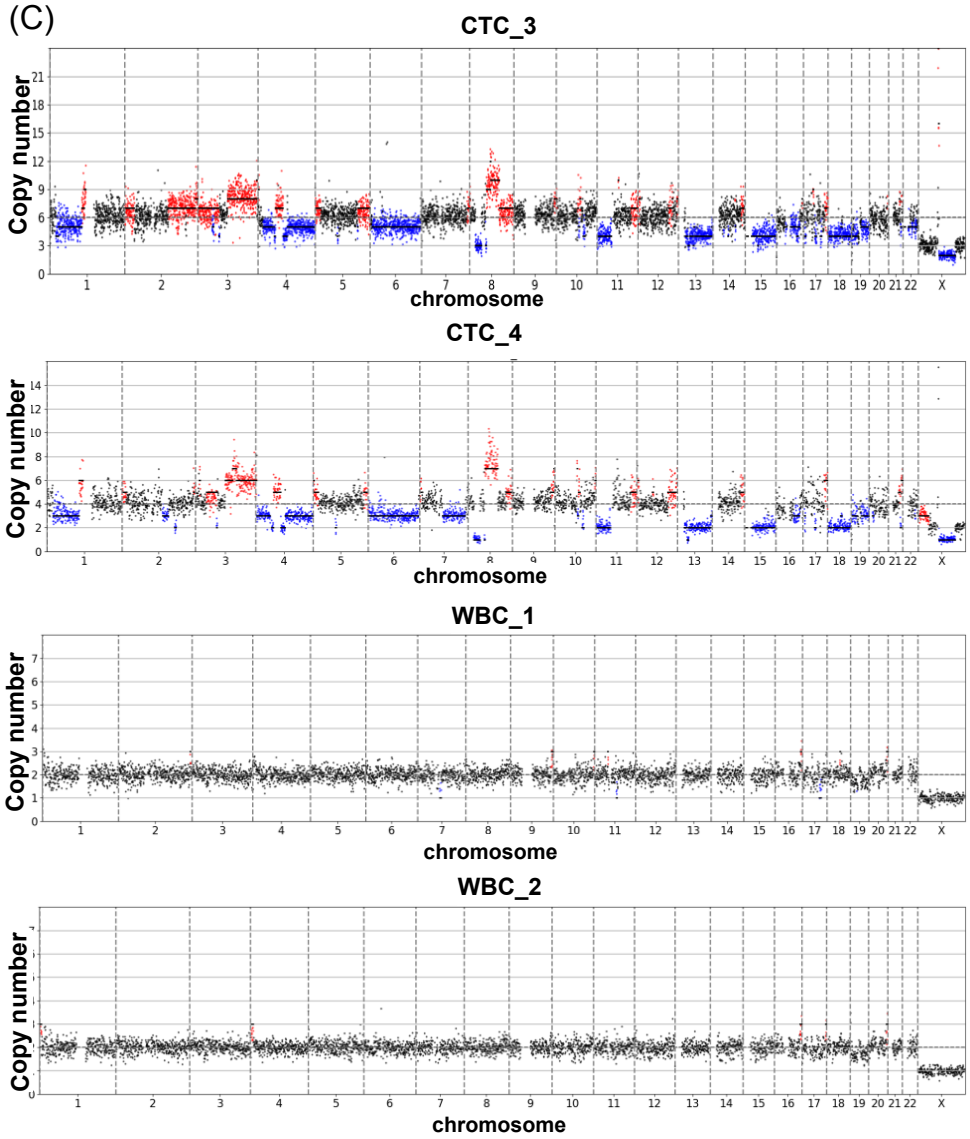
(A)



(B)

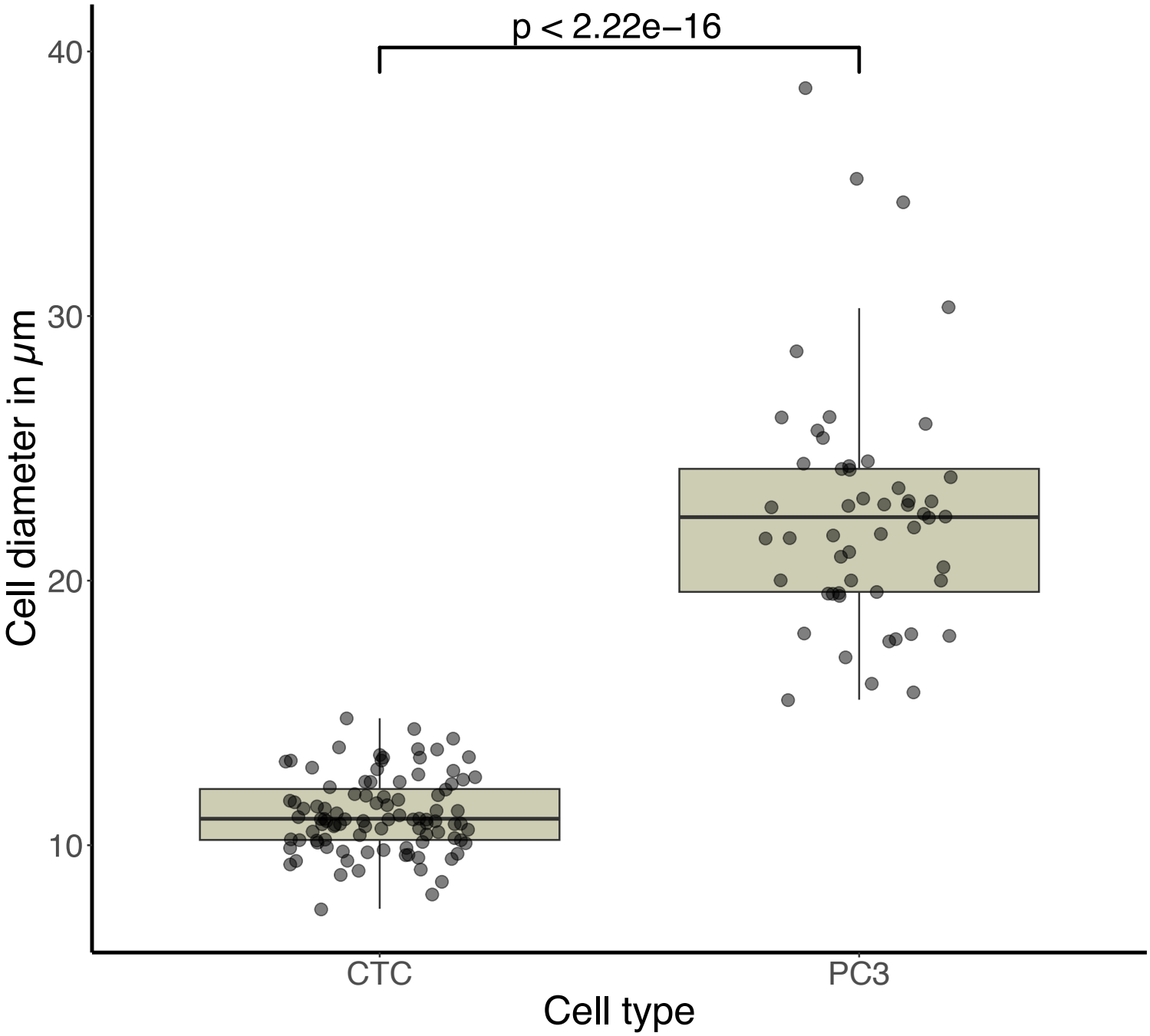
	CTC_1	CTC_2	CTC_3	CTC_4	WBC_1	WBC_2	WBC_3
AR	24	17	16	14	1	1	1
MYC	6	6	7	5	2	2	2
FGF1	4	5	7	4	2	2	2
KLK3	3	3	5	3	2	2	2
NKX3-1	2	1	3	1	2	2	2
PIK3CA	5	8	8	6	2	2	2
BRCA-2	1	1	3	1	2	2	2
GSK3B	5	8	8	6	2	2	2
CTBP1	3	5	7	6	2	2	2

(C)



Supplementary Figure 5. Single CTC genomic analysis.

(A) WGA product suitable for genomic CNA analysis estimated with Ampli1™ QC kit. 3 bands represent adequate genomic quality, and 4 bands represent optimal quality. Lane 1-4: putative CTCs. Lane 5-7: WBCs. Lane 8-9: Negative WGA control (sterile DPBS) and positive WGA control (1 ng/µl DNA). Lane 10-11: Negative QC control (sterile H_2O) and positive QC control (100 ng/µl DNA) (B) Heatmap of absolute CN shown for a curated set of prostate cancer-associated genes for each CTC and WBC. Neutral: colorless; loss: blue; gain: red. (C) CNA profiles of analyzed single CTCs and WBCs. Black points along a dashed line depict the main ploidy. Allele amplification and loss are depicted as red and blue points, respectively. Y-axis maximum is set to 4 times main ploidy. Y-chromosome is excluded.



Supplementary Figure 6. Size comparison of patient-derived CTCs and the PC3 prostate cancer cell line

The size of the cells were measured based on the average fluorescence intensity of FITC (anti-CK conjugated to FITC) using the Gen5 software (Biotek, v. 3.10). Only cells with aver-age fluorescence intensities > 10.000 for CK and DAPI were included in the analysis. None of the cells were positive for anti-CD45 conjugated to APC. 96 $CK^+/CD45^-/DAPI^+$ patient-derived CTCs were included in the analysis along with 52 $CK^+/CD45^-/DAPI^+$ PC3 prostate cells spiked into blood. Statistical analysis was performed in R (v 4.2.1) using a non-parametric Mann-Whitney *U* test.

Supplementary table 1. Sensitivity of the assay using PC3 cells spiked into 10 mL of healthy donor blood

Sample	Cells spiked in	Cells detected	Recovery (%)	Harvested cell number
10 cells	11	9	82	87418
10 cells	11	3	27	62870
10 cells	6	6	100	110630
10 cells	6	2	33	40710

Supplementary table 2. Cell characterization and size distribution of CK^+ prostate cancer cell lines

Cell lines	DU145	C4-2	LNCaP	PC3
Cell count (CK+ population)	1571	3636	3006	3881
Area μm^2, mean (sd)	460 (±101.2)	536 (±134.8)	401 (±84.9)	542 (±124.9)
Area μm^2, range	252 - 1104	246 - 938	247 - 743	276 - 1352
Diameter µm, mean (sd)	24 (± 2.6)	26 (± 3.2)	22.5 (± 2.3)	26 (± 2.8)
Diameter, range (µm)	17.9 - 37.5	17.7 - 34.6	17.7 - 30.8	18.8 - 41

Abbreviations: sd, standard deviation, CK, cytokeratin

Supplementary table 3. Sequencing Coverage and Quality Statistics

Sample ID	Sequenced reads per sample	Total number of called cells[a]	Number of uniquely mapped reads per called cell[b]	Coverage per targeted base per called cell[c]	DLRS[d]	R50[e]
CTC_1	2,105,265	1	1,992,341	0.10	0.24	47
CTC_2	2,664,722	1	2,521,727	0.13	0.26	47
CTC_3	2,873,050	1	2,716,183	0.14	0.23	47
CTC_4	1,661,837	1	1,572,124	0.08	0.24	47
WBC_1	1,721,013	1	1,618,971	0.08	0.22	47
WBC_2	1,556,310	1	1,464,792	0.08	0.22	47
WBC_3	1,643,096	1	1,550,648	0.08	0.21	47

[a] Data was aligned to Homo sapiens hg19 reference sequence.

[b] After quality control and filtering for mapping quality > 5 to exclude multi-mapping reads.

[c] The analysis was based on whole genome sequencing. Suitable base pairs for analysis, after removal of centromers, telomers and highly repetitive low complexity regions, were set to 2,869,277,400 bp.

[d] To calculate the derivative log ratio spread (DLRS), a measure of point-to-point copy number signal consistency, 200,000 randomly sampled reads were extracted. The copy number is calculated based on 1 Mbp window (CN) and a main ploidy of 2. Lastly, the DLRS is calculated as below, where diff. is defined as the n-th discrete difference between consecutive windows along the genome.

$$DLRS = \text{std}\left(\frac{diff\left(log2\left(\frac{CN}{2}\right)\right)}{\sqrt{2}}\right)$$

[e] The percentage of target (covered by Ampli1 fragments) covered by 50% of reads (R50), a measure of WGA-library complexity ranging from 0 to 50, with larger values indicating higher complexity, was calculated on 200,000 randomly sampled reads as follows:

$$R50 = \frac{N_{100000}}{N_{200000}} \times 100$$

In this N is defined as the number of *Ampli1* fragments, sorted from most covered, covered by 100,000 or 200,000 reads.