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Simulations: cell capture in the microfluidic device

Cell capture occurs when the magnetic force acting on the cells counterbalances the drag force exhibited by the flow. Maxwell's equation can be used to determine the magnetic flux density.

$$\boldsymbol{F = \frac{B^2 A}{2\mu_0}}$$

Where, F is the pull-force (N) and equals to 0.38 lbs for 2 magnets.

B is the magnetic flux (T)

A is the area at the surface of one of the poles (m^2) (D=1/16" & L=1/4")

μ_0 is the permeability of free space, ($4\pi \times 10^{-7}$)

The magnetic force acting on the cell containing magnetic nanoparticles can be calculated using the following formula:

$$\vec{\boldsymbol{F}}_m = N_b V_m \frac{\Delta\chi_{bead}}{\mu_0}(\vec{\boldsymbol{B}} \cdot \nabla)\vec{\boldsymbol{B}}$$

Where N_b is the number of beads per cell, V_m is the bead volume, $\Delta\chi_{bead}$ [unitless] represents the difference between the magnetic susceptibility of the bead and the medium, μ_0 [H/m] is the permeability of free space ($4\pi \times 10^{-7}$ H/m), and B [T] is the applied magnetic field. Here we used magnetic beads with diameter of 100 nm.

Stokes' law can be used to determine the transverse drag force acting on a cell, neglecting wall effects at low Reynolds numbers, according to formula (II):

$$\vec{\boldsymbol{F}}_d = -6\pi\eta r\vec{v}$$

Where r [m] is the cell radius (10 μm), η [Pa×s] is the dynamic viscosity of the medium (0.001 Pa×s), and v [m/s] is the velocity of the cell.

We defined the perimeter of the capture regions around the X structures by the most significant decrease in the magnitude of the flow velocity. The flow velocity at the edge these areas are used to calculate a maximum threshold for the drag force required to capture a cell. The simulated flow rate was set at 2 mL h^{-1}. The minimum number of beads required for cell capture at each of the 8 zones was calculated and the results are provided in Table S1.

Supplementary Table 1. Calculation of the minimum number of magnetic beads required for cell capture

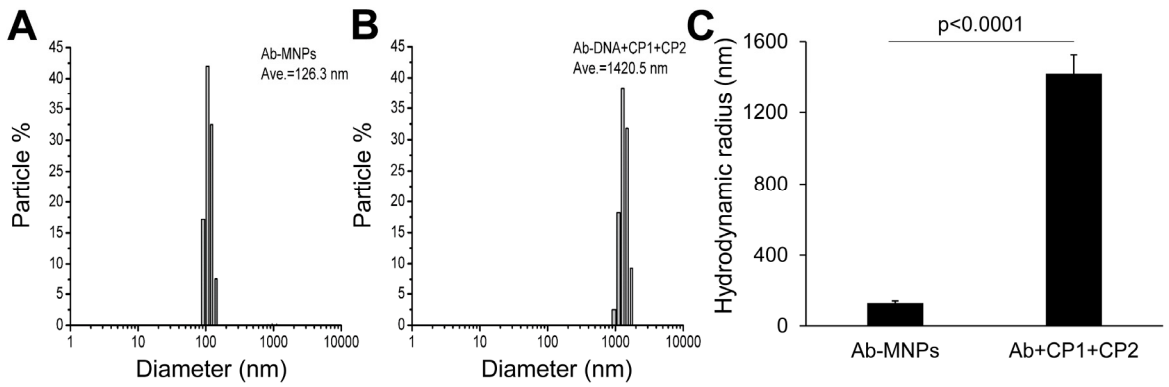
Zone	Flow velocity threshold at capture site (m/s)	Drag force (N)	Minimum number of beads required for cell capture	Estimated number of targeted protein copies
1	7.84E-07	-8.87E-14	2.23E+04	2794
2	4.04E-07	-4.57E-14	1.15E+04	1440
3	2.52E-07	-2.85E-14	7.19E+03	899
4	2.12E-07	-2.40E-14	6.04E+03	755
5	1.82E-07	-2.06E-14	5.19E+03	649
6	1.42E-07	-1.60E-14	4.04E+03	505
7	1.21E-07	-1.37E-14	3.45E+03	432
8	1.01E-07	-1.14E-14	2.88E+03	361

Supplementary Table 2. The sequence of nucleic acids used in the experiments

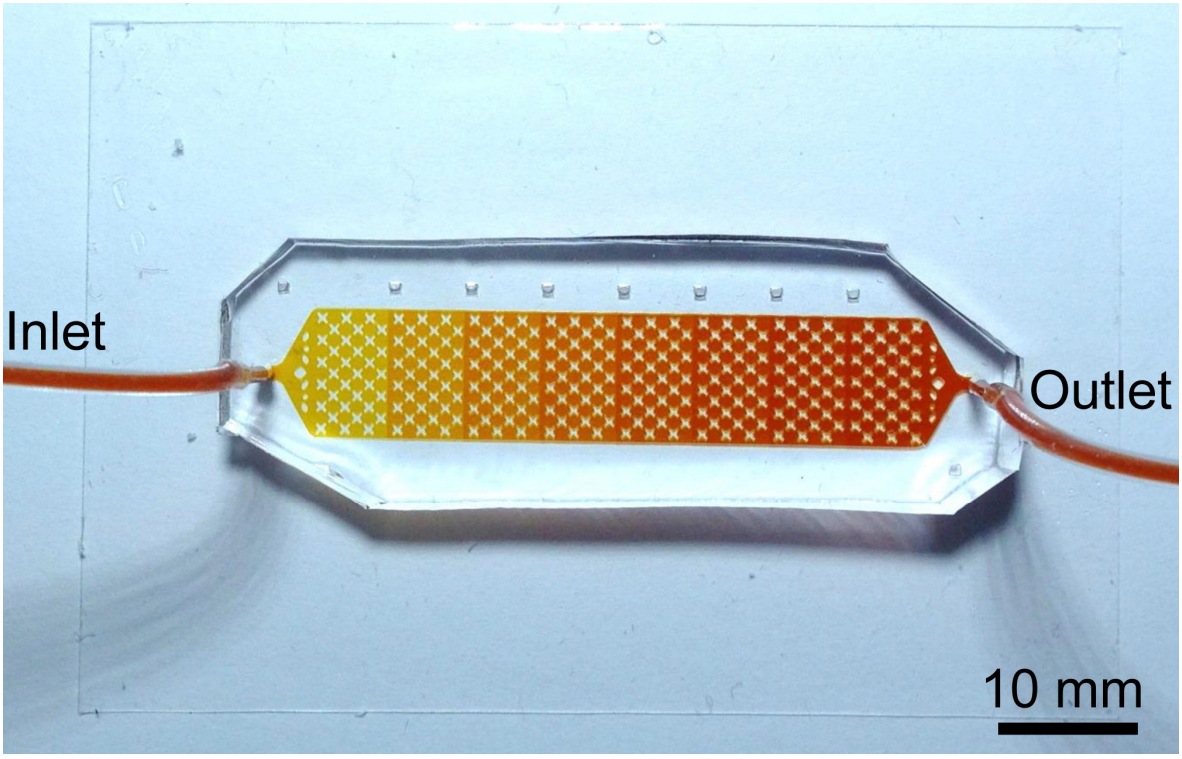
Nucleic acid	Sequence
ssDNA	5' AGT CTG GCA TGA CTG AGA CAT AGG ATG GAT TAC CAT CCA TAG GAT 3'–(TEG) –Biotin
CP1	Biotin–(TEG) –5' ATC CTA TGG ATG GTA ATC CA 3'
CP2	5' TGT CTC AGT CAT GCC AGA CT 3'–(TEG) –Biotin
CP1-AF488	Biotin–(TEG) –5' ATC CTA TGG ATG GTA ATC CA 3'–AF488
AF488-CP2	AF488–5' TGT CTC AGT CAT GCC AGA CT 3'–(TEG) –Biotin
c-Myc FP	5' AAA CAC AAA CTT GAA CAG CTA C 3'
c-Myc RP	5' ATT TGA GGC AGT TTA CAT TAT GG 3'
vimentin FP	5' GCA AAG ATT CCA CTT TGC GT 3'
vimentin RP	5' GAA ATT GCA GGA GGA GAT GC 3'
GAPDH FP	5' CTT CAC CAC CAT GGA GGA GGC 3'
GAPDH RP	5' GGC ATG GAC TGT GGT CAT GAG 3'

Supplementary Table 3. The analytical performance of the therapeutic protein analysis approach benchmarked against FACS-based microfluidic Western blotting

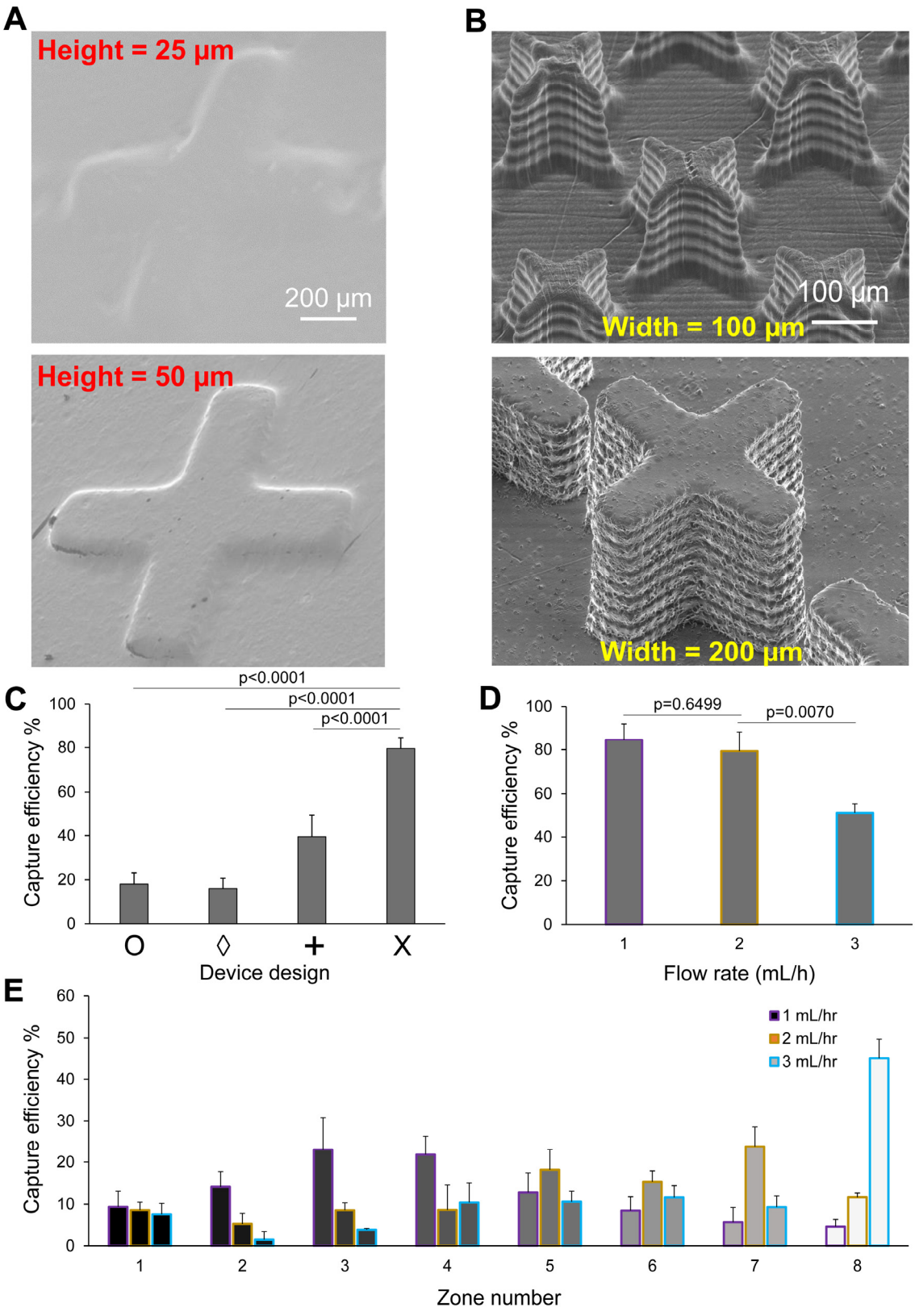
	FACS-based microfluidic Western blotting [1]	Therapeutic protein analysis approach
Sample preparation	- Cells are first sorted using FACS then aliquoted to the scWestern microwells. Cells are then lysed and analysed for several proteins. - Clinical specimens require pre-enrichment using a size- and deformability-selective microfluidic device (Vortex-HT chip)	- Cells are fixed, permeabilized, incubated with the probes then loaded into the microfluidic device. Cell lysis is not required. - Clinical specimens require pre-enrichment using Ficoll tubes
Assay time	~4 hours	~6 hours
Cost	N/A	$10 per device
Sensitivity	200 cells	10 cells
Specificity	High because it includes electrophoresis/isoelectric focusing step in addition to immunoprobing of the target	High because it includes an identification step for the protein-expressing cells in addition to immunoprobing of the target
Dynamic range	200 – 1000 cells	10 – 500 cells
Multiplexing	11 proteins after stripping and re-probing	1 protein per cell but any number of proteins can be analysed in parallel.



Supplementary Figure 1. Dynamic light scattering (DLS) measurements. (**A**) DLS analysis of the hydrodynamic radius of a c-Myc specific antibody modified directly with MNPs via biotin-streptavidin coupling vs. (**B**) c-Myc antibody modified with ssDNA and subsequently hybridized with MNPs-labelled CP1 and MNPs-labelled CP2. (**C**) A comparison between the hydrodynamic radius measured for (A) and (B). Error bars represent the s.d. of biological replicates (N=3). Unpaired two-tailed Student's *t*-test was used to the analyse the data in (**C**).

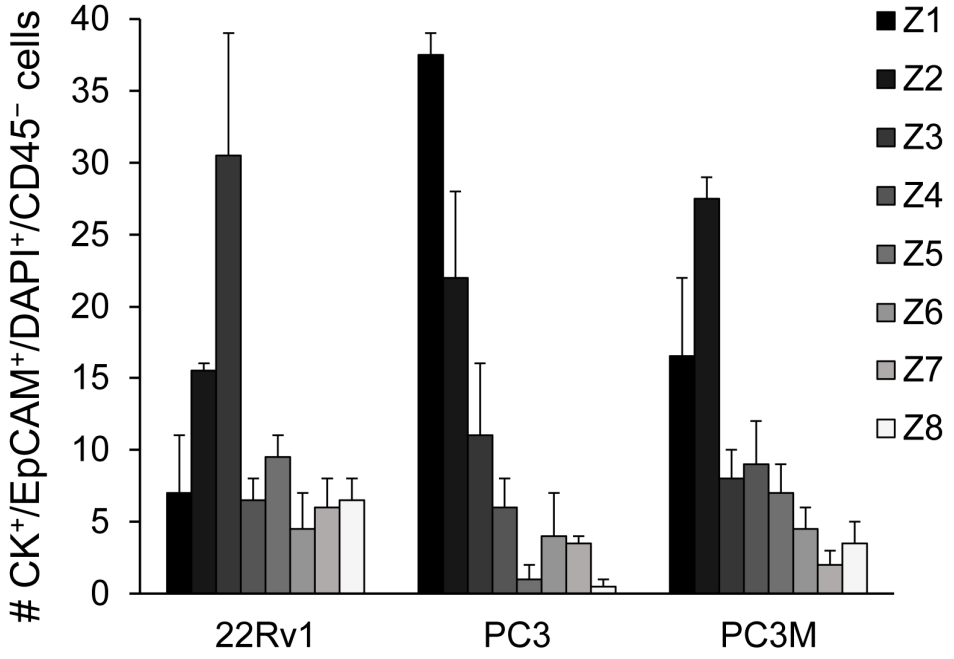


Supplementary Figure 2. Microfluidic device used for intracellular protein analysis. Two arrays of magnets were positioned on the upper and lower sides of the device to capture cells containing trapped aggregates of magnetic nanoparticles.

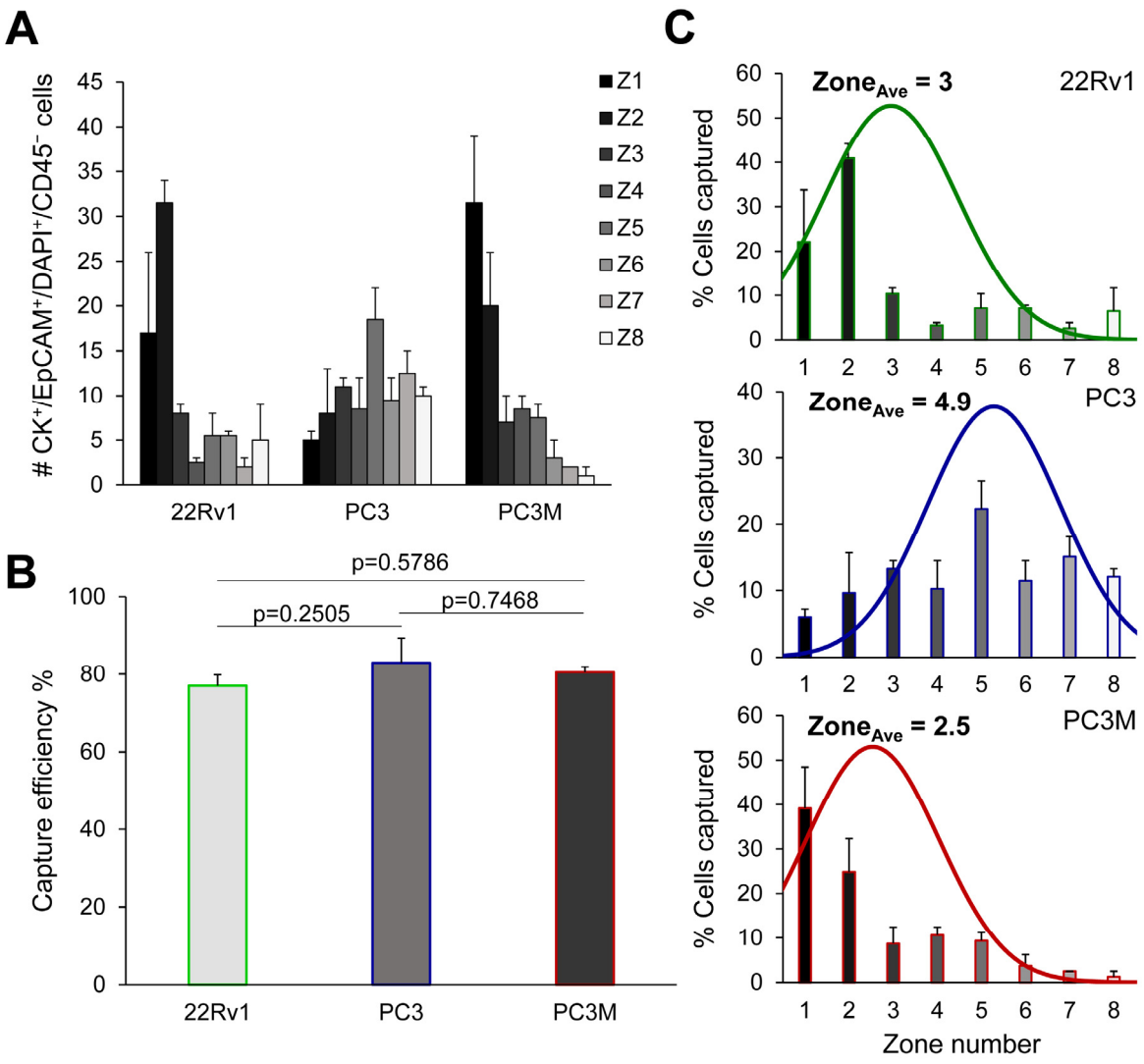


Supplementary Figure 3. Optimization of the device design and operational flow rate. (**A**) SEM images of the microfabricated structures printed at two different heights, including 25 µm and 50 µm. Only a height of 50 µm can be printed robustly. (**B**) SEM images of the microfabricated structures printed at two different widths, including 100 µm and 200 µm. Only a width of 200 µm can be printed

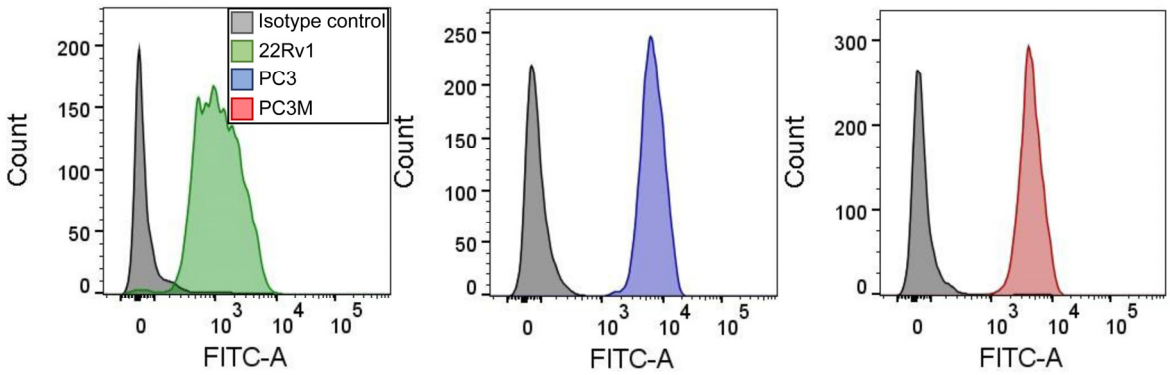
without significant defects. The length of the device is 5.7 cm, which is limited by the maximal printable length of the printer. (**C**) Optimization of the device design. We studied the capture efficiency of LNCaP cells using microfluidic devices that were designed using microfabricated structures with different shapes, including O, ◊, +, X. (**D**) Optimization of the flow rate within the device. We studied the capture efficiency of PC3 cells at different flow rates, including 1, 2, and 3 mL h^{-1}. (**E**) Distribution of PC3 cells among the 8 capture zones. Prior to experiments, the cells were incubated with MNPs-tagged EpCAM antibody. The cells were loaded into the microfluidic device, fixed, permeabilized, and stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. The capture efficiency was determined by dividing the number of captured cells by the total number of cells loaded into the device. Only 50 cells were used in these trials. Error bars represent the s.d. of biological replicates (N=4). Ordinary one-way ANOVA tests were used to analyse the data in (C) and (D) and Turkey's corrections with 95% confidence intervals and significance were used.



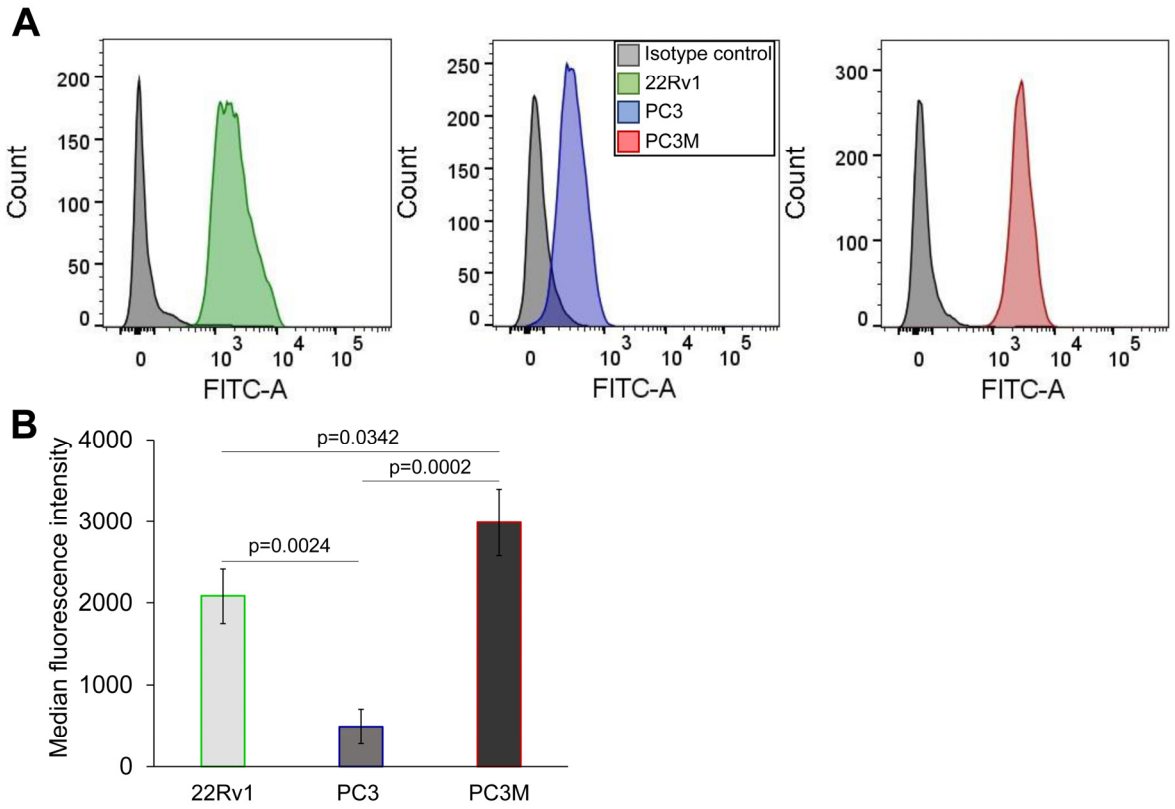
Supplementary Figure 4. Raw data for c-Myc analysis in prostate cancer cell lines tested in Figure 2. The cells were fixed with 4% PFA, permeabilized with 0.3% TX-100, and incubated with a c-Myc specific antibody modified with ssDNAs that were subsequently hybridized with MNPs-labelled CP1 and MNP-labelled CP2. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1}, stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. One hundred cells were used in these trials. Error bars represent the s.e.m. of biological replicates (N=3).



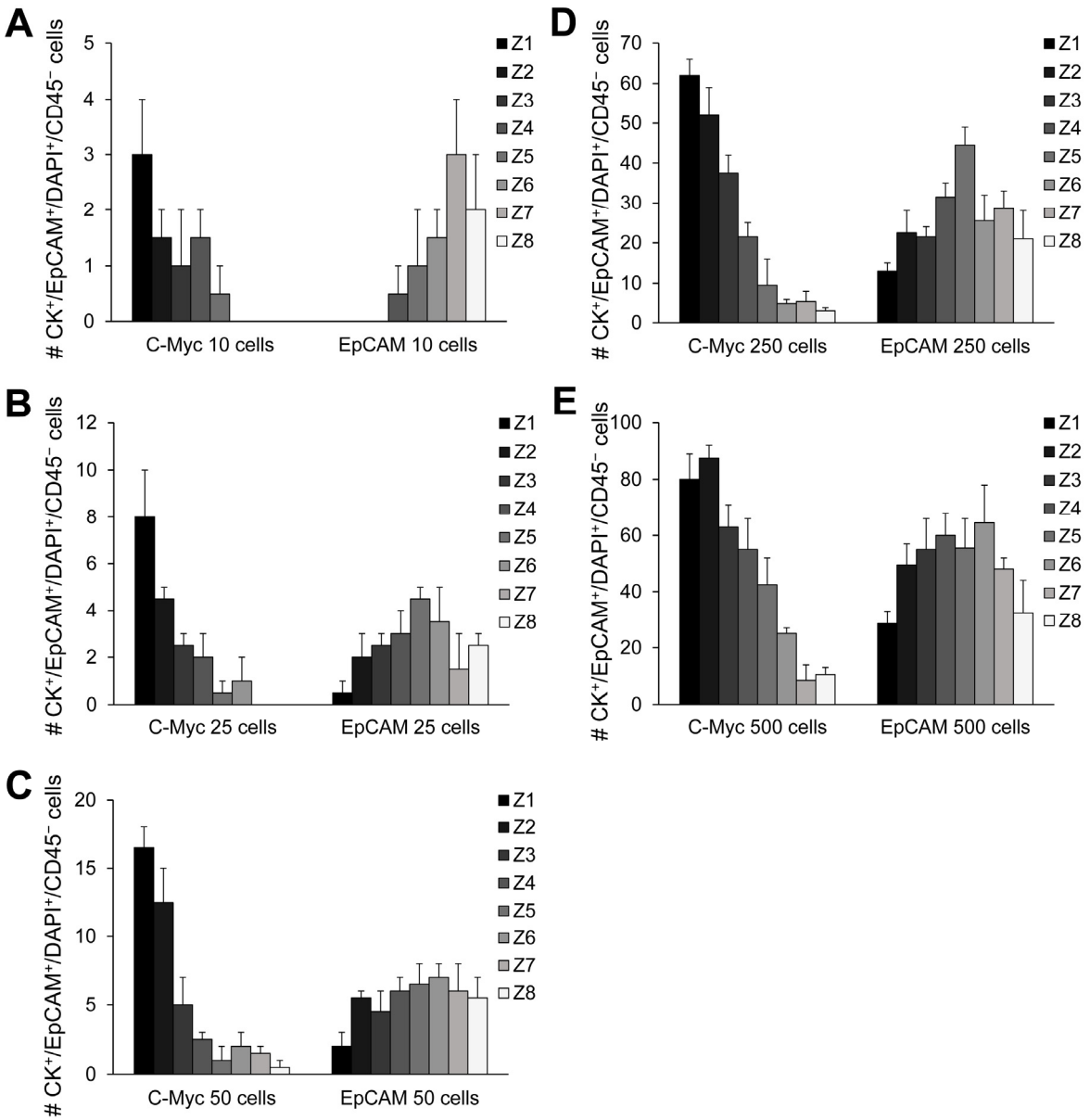
Supplementary Figure 5. Analysis of EpCAM expression in prostate cancer cell lines using the microfluidic approach. (**A**) Three prostate cancer cell lines, including 22Rv1, PC3, and PC3M, were incubated with MNPs-tagged EpCAM antibody. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1}, stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. (**B**) Capture efficiency determined for each cell line by dividing the number of captured cells by the total number of cells loaded into the device. One hundred cells were used in these trials. (**C**) Determination of the average capture zone ($Zone_{Ave}$) for 22Rv1, PC3, and PC3M cells. The curves represent the normal distribution fit to the data. Cells with high EpCAM expression (i.e. PC3M) are mainly captured in earlier zones and thus have lower $Zone_{Ave}$ value compared to cells exhibiting low EpCAM expression (i.e. PC3). Error bars represent the s.d. of biological replicates (N=3). Ordinary one-way ANOVA tests were used to analyse the data in (**B**) and Turkey's corrections with 95% confidence intervals and significance were used.



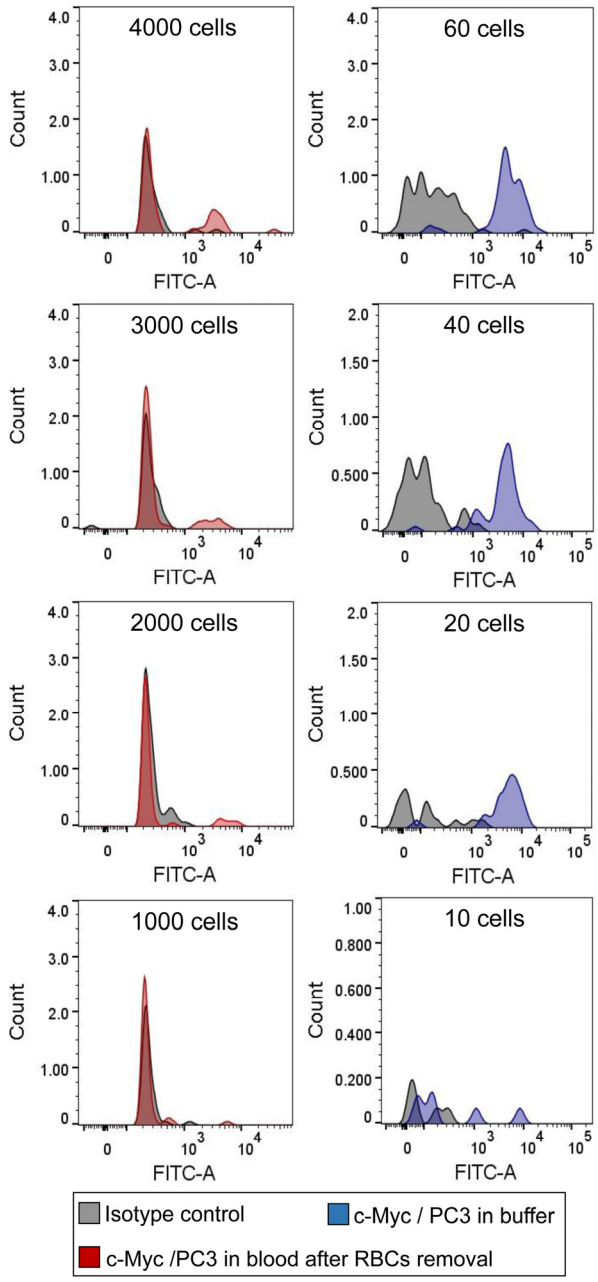
Supplementary Figure 6. Flow cytometric analysis of c-Myc expression in three prostate cancer cell lines. Prior to analysis, cells were fixed with 4% PFA, permeabilized with 0.2% TX-100, and incubated with c-Myc specific antibody for 30 min at room temperature. After washing, the cells were incubated FITC-labelled rabbit antibody for 30 min at room temperature. A Control experiment was carried out using an isotype antibody.



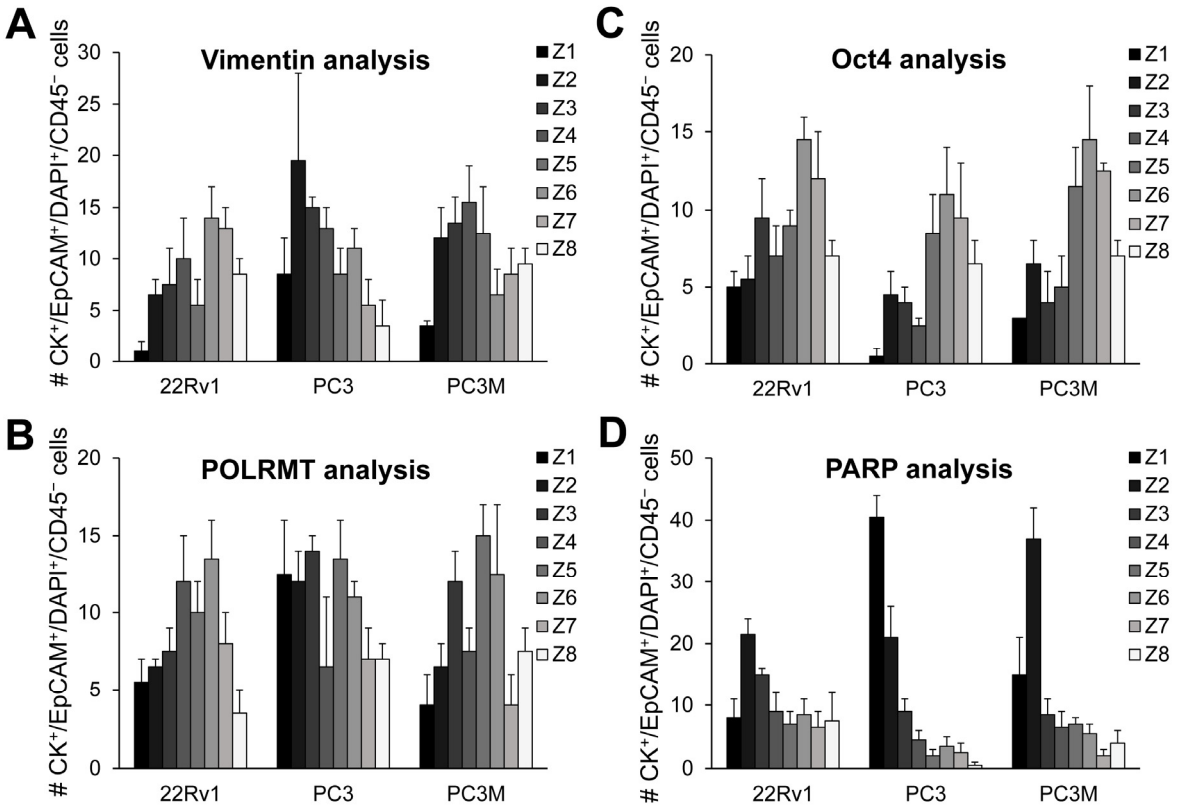
Supplementary Figure 7. Flow cytometric analysis of EpCAM expression in three prostate cancer cell lines. (**A**) Flow cytometry histograms and (**B**) median fluorescence intensity are shown. Prior to analysis, cells were fixed with 4% PFA and incubated with EpCAM specific antibody for 30 min at room temperature. After washing, the cells were incubated FITC-labelled rabbit antibody for 30 min at room temperature. A Control experiment was carried out using an isotype antibody. Error bars represent s.d. of 3 biological replicates. Ordinary one-way ANOVA tests were used to analyse the data and Turkey's corrections with 95% confidence intervals and significance were used.



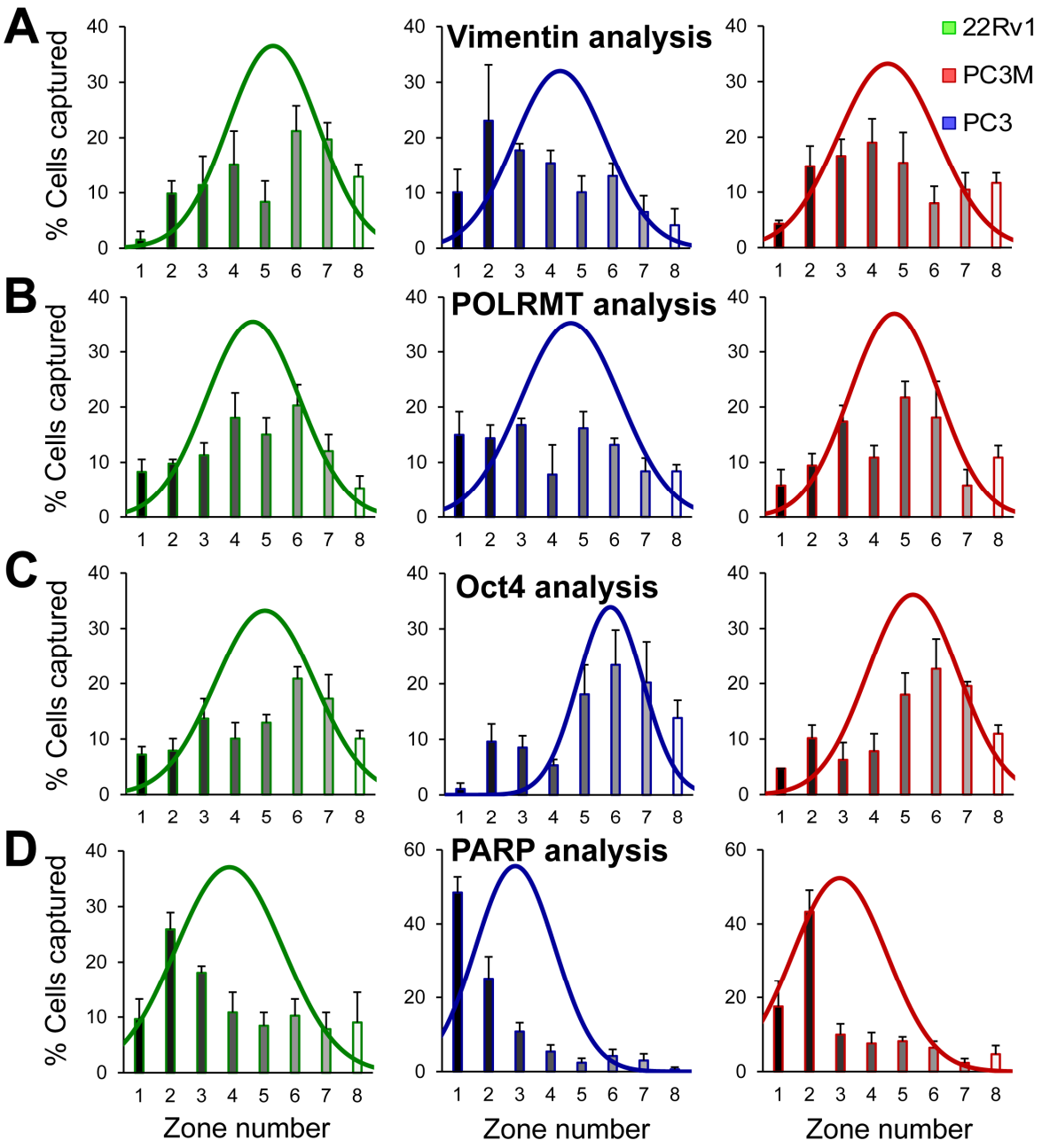
Supplementary Figure 8. Raw data for the sensitivity and dynamic range of the method tested in Figure 2. The sensitivity of the approach was tested by spiking different number of PC3 cells into 1 mL of blood. Prior to analysis, the RBCs were removed using the Ficoll method. The cells were fixed with 4% PFA, permeabilized with 0.3% Tx-100, and incubated with a c-Myc specific antibody modified with ssDNAs that were subsequently hybridized with MNPs-labelled CP1 and MNPs-labelled CP2. Another experiment was carried out in which the cells were captured using MNPs-labelled EpCAM antibody. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1}, stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. Error bars represent the s.e.m. of biological replicates (N=3).



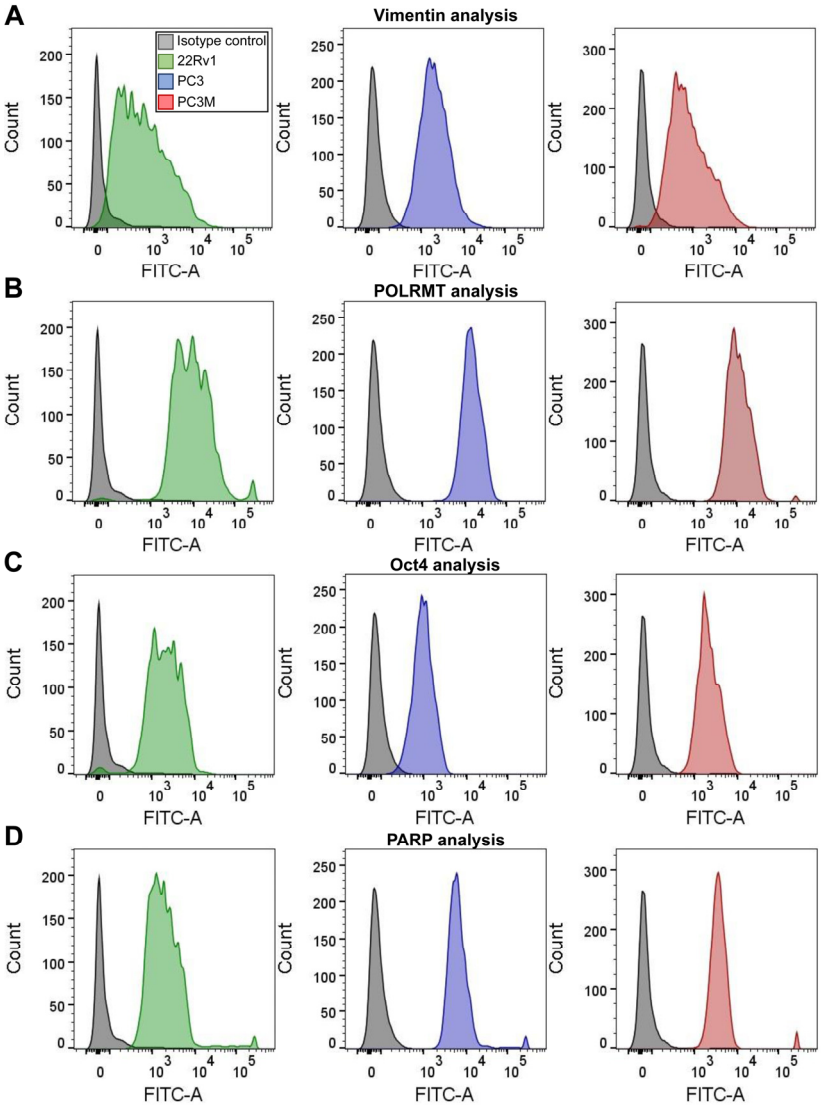
Supplementary Figure 9. Sensitivity of the flow cytometric analysis of c-Myc in PC3 cells. PC3 cells were spiked into PBS buffer (right panel) and whole blood (left panel). The RBCs were removed using the Ficoll method. The cells were fixed with 4%, permeabilized with 0.2% TX-100, and incubated and incubated with c-Myc specific antibody for 30 min at room temperature. After washing, the cells were incubated FITC-labelled rabbit antibody for 30 min at room temperature. A control experiment was carried out using an isotype antibody. Importantly, c-Myc was detected in the buffer solution at a low cell count, however a high cell count was needed for c-Myc detection in blood even after RBCs removal.



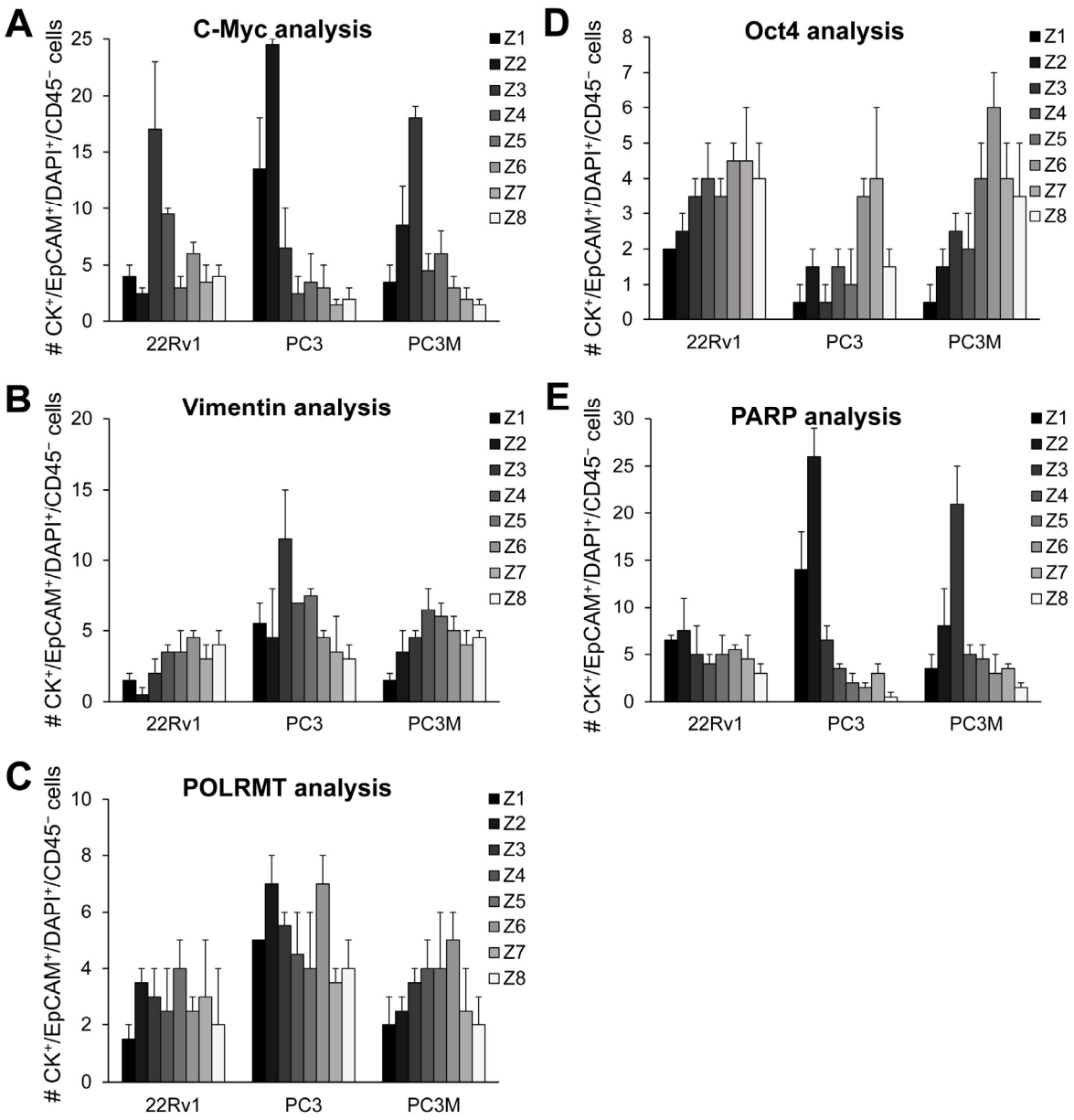
Supplementary Figure 10. Raw data for the analysis of clinically relevant intracellular proteins tested in Figure 3. Four proteins were analysed, including (**A**) vimentin, (**B**) POLRMT, (**C**) Oct4, and (**D**) PARP1 in 3 prostate cancer cell lines. Prior to analysis, the cells were fixed with 4% PFA, permeabilized with 0.3% TX-100, and incubated with a specific antibody modified with ssDNAs that were subsequently hybridized with MNPs-labelled CP1 and MNPs-labelled CP2. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1}, stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. One hundred cells were used in these trials. Error bars represent the s.e.m. of biological replicates (N=3).



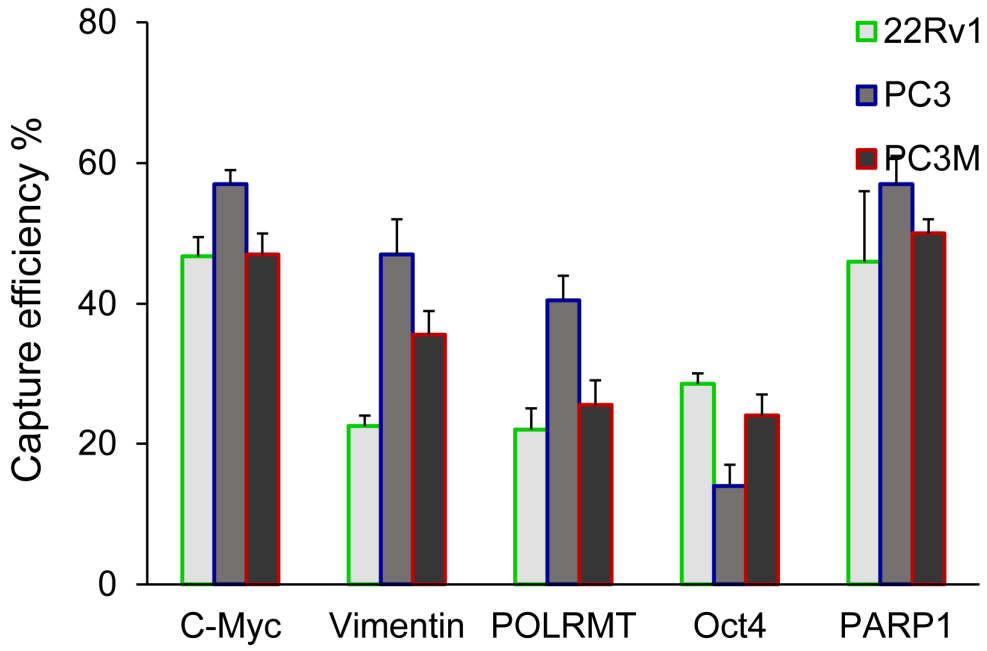
Supplementary Figure 11. Determination of the median capture zone after targeting clinically relevant intracellular tumour proteins. Analysis of (**A**) vimentin, (**B**) POLRMT, (**C**) Oct4, and (**D**) PARP1, in 22Rv1, PC3, and PC3M cell lines using the new approach. The curves represent the normal distribution fit to the data. One hundred cells were used in these trials. Error bars represent the s.e.m. of biological replicates (N=3).



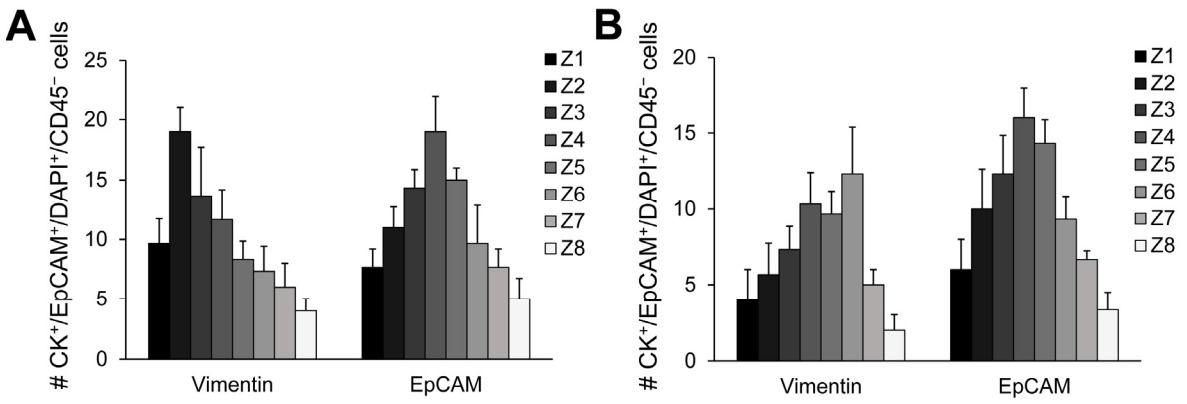
Supplementary Figure 12. Flow cytometric analysis of clinically relevant intracellular tumour proteins. Analysis of (**A**) vimentin, (**B**) POLRMT, (**C**) Oct4, and (**D**) PARP1, in 22Rv1, PC3, and PC3M cell lines. Prior to analysis, cells were fixed with 4% PFA, permeabilized with 0.2% TX-100, and incubated with either vimentin, POLRMT, Oct4, or PARP1 specific antibody for 30 min at room temperature. After washing, the cells were incubated FITC-labelled rabbit antibody for 30 min at room temperature. A control experiment was carried out using an isotype antibody.



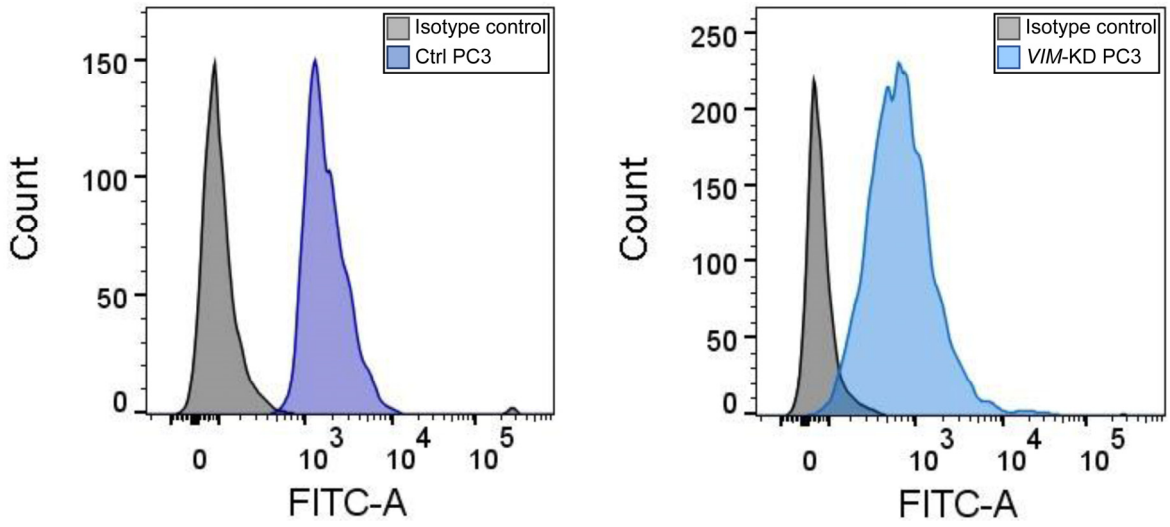
Supplementary Figure 13. Cell capture after targeting the intracellular proteins directly with specific antibodies conjugated with magnetic nanoparticles. Three prostate cancer cell lines, including 22Rv1, PC3, and PC3M, were fixed with 4% PFA, permeabilized with 0.3% Tx-100, and incubated with MNPs-labelled antibodies specific to (**A**) c-Myc, (**B**) vimentin, (**C**) POLRMT, (**D**) Oct4, and (**E**) PARP1. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1}, stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. One hundred cells were used in these trials. Error bars represent the s.e.m. of biological replicates (N=3).



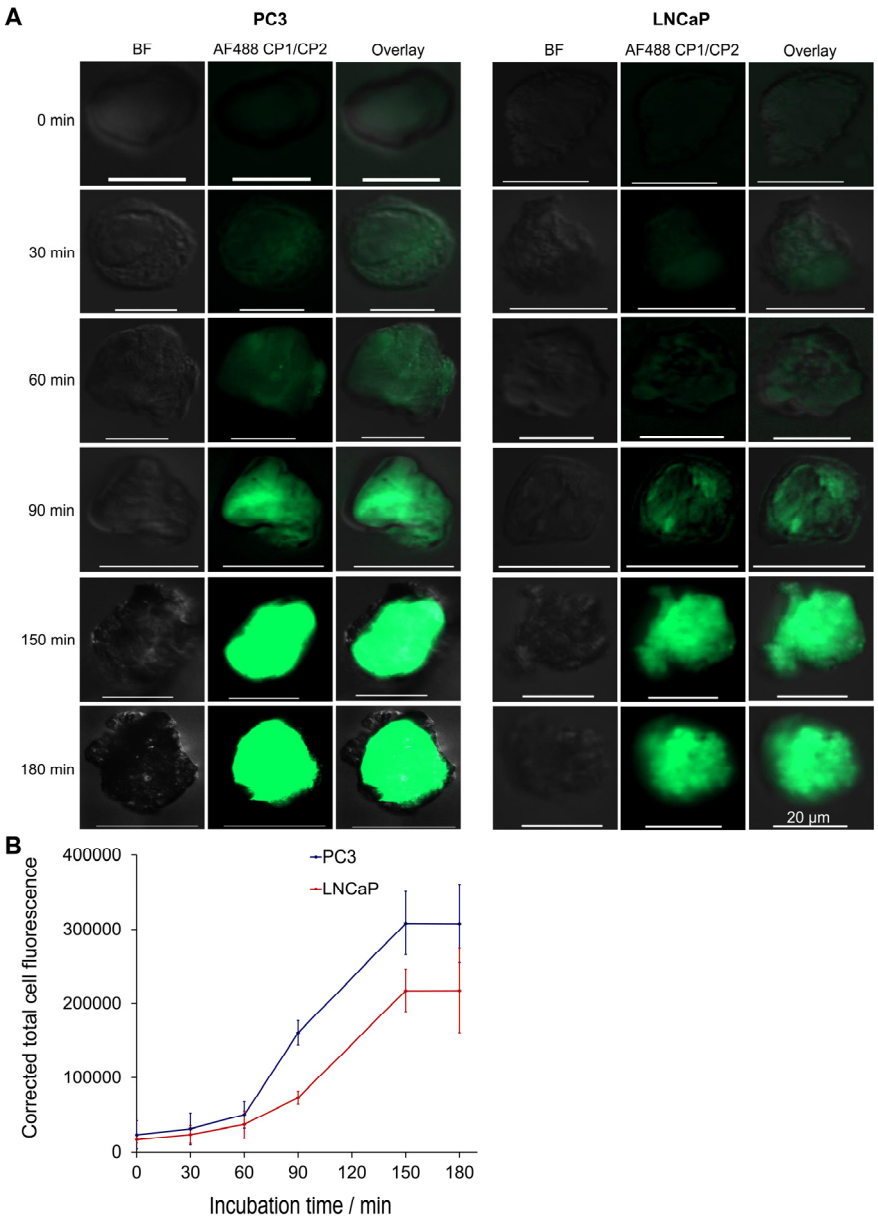
Supplementary Figure 14. Capture efficiency of prostate cancer lines by targeting the intracellular proteins directly with specific antibodies conjugated with magnetic nanoparticles. Three prostate cancer cell lines, including 22Rv1, PC3, and PC3M, were fixed with 4% PFA, permeabilized with 0.3% TX-100, and incubated with MNPs-labelled antibodies specific to either c-Myc, vimentin, POLRMT, Oct4, or PARP1. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1}, stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. One hundred cells were used in these trials. Error bars represent the s.e.m. of biological replicates (N=3).



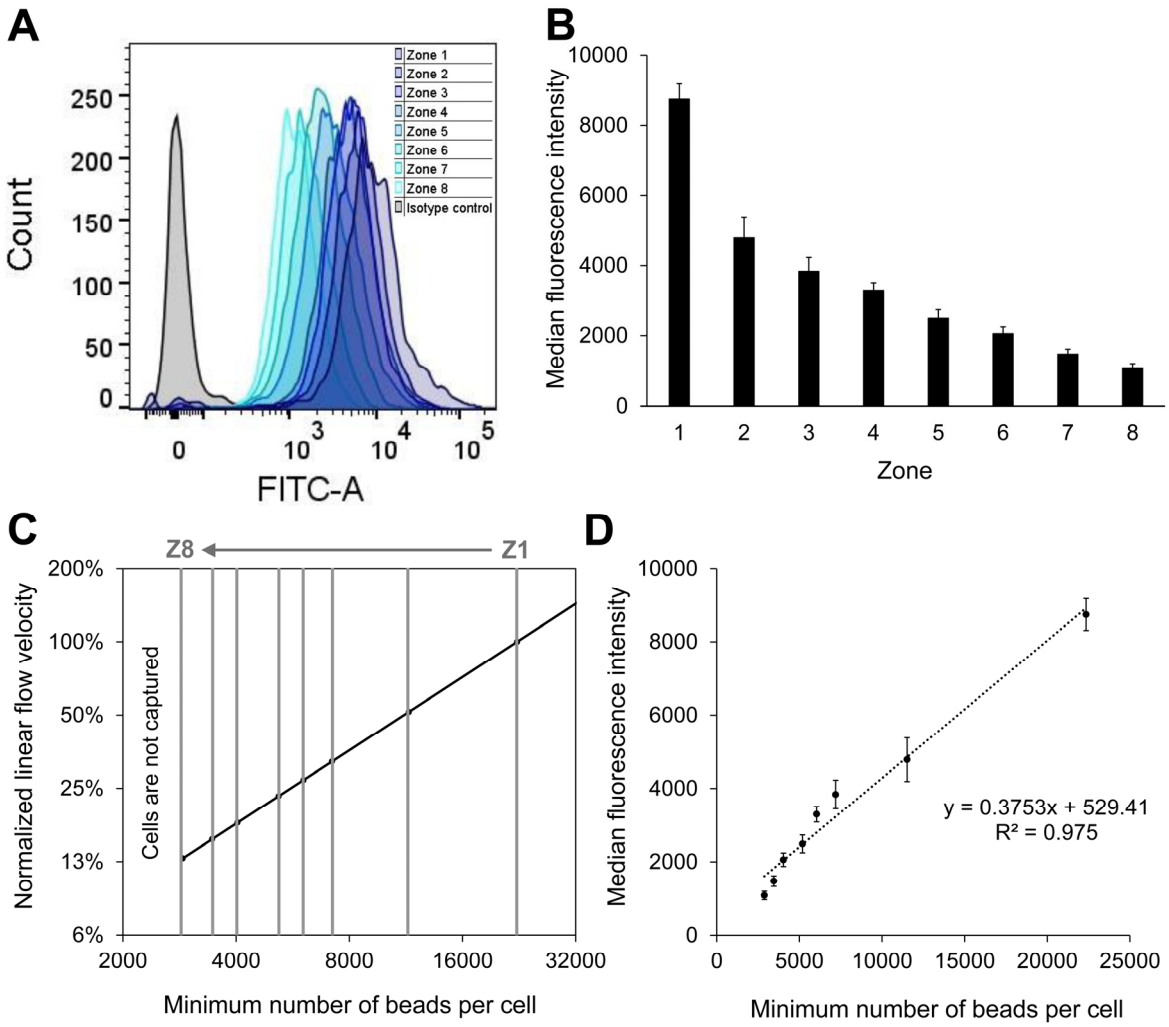
Supplementary Figure 15. Raw data for the analysis of vimentin protein in PC3 cells after knocking down the *VIM* gene tested in Figure 3. Prior to analysis, the cells were fixed with 4% PFA, permeabilized with 0.3% TX-100, and incubated with a specific antibody modified with ssDNAs that were subsequently hybridized with MNPs-labelled CP1 and MNPs-labelled CP2. The total count of cells analysed was determined by targeting EpCAM using MNPs-labelled EpCAM antibody. (**A**) A control experiment was carried out using PC3 cell previously incubated with nontargeting siRNAs in the same delivery medium. (**B**) PC3 cells were incubated with *VIM* specific siRNAs in the delivery medium. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1}, stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. One hundred cells were used in these trials. Error bars represent the s.d. of biological replicates (N=3).



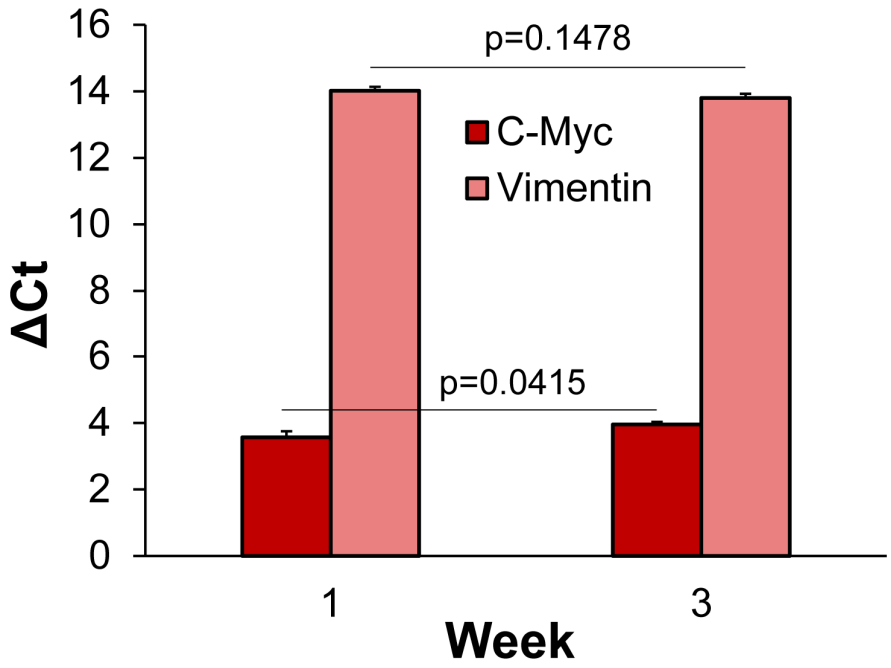
Supplementary Figure 16. Flow cytometric analysis of vimentin protein in PC3 cells after knocking down the *VIM* gene. Vimentin protein was analysed in PC3 cells after knocking down the *VIM* gene using specific siRNAs. A control experiment was carried out in which PC3 cells were incubated with nontargeting siRNAs under the same experimental conditions. Prior to analysis, cells were fixed with 4% PFA, permeabilized with 0.2% TX-100, and incubated with vimentin specific antibody for 30 min at room temperature. After washing, the cells were incubated FITC-labelled rabbit antibody for 30 min at room temperature. A Control experiment was carried out using an isotype antibody.



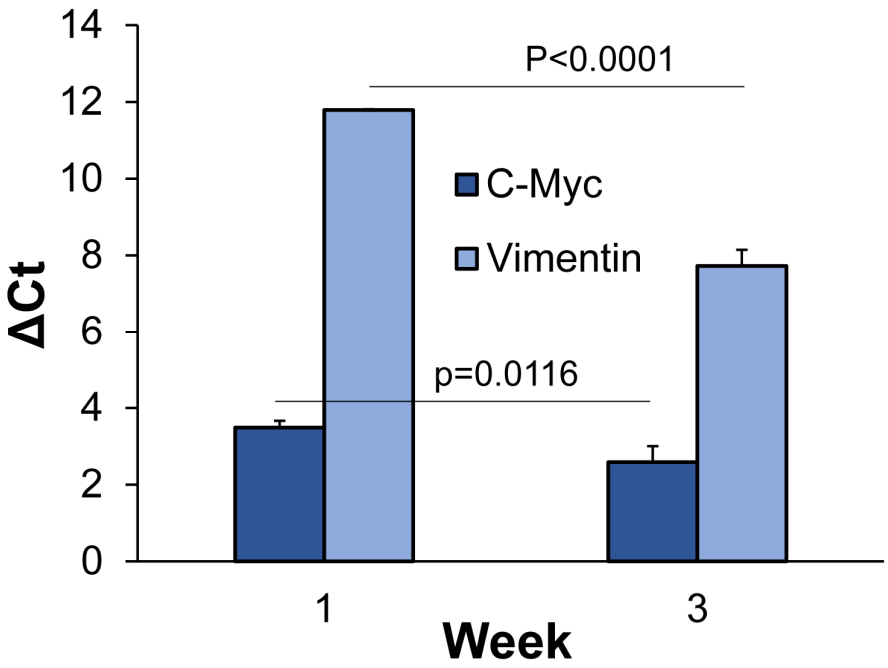
Supplementary Figure 17. FISH analysis of the rate of entry of capture probes into prostate cancer cells. (**A**) PC3 and LNCaP cells were fixed with 4% PFA, permeabilized with 0.3% TX-100, and incubated with a vimentin specific antibody modified with ssDNAs that were subsequently hybridized with MNPs-labelled CP1 and MNPs-labelled CP2 for 0, 30, 60, 90, 150, 180 min. The capture probes were dually labelled with MNPs at one end and AF488 at the other end. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1}. One hundred cells were used in these trials. (**B**) Quantitation of cellular fluorescence of captured cells as a function of the incubation time with MNPs-labelled CP1 and MNPs-labelled CP2. Values are corrected for background fluorescence. Error bars represent the s.d. of technical replicates (N=3).



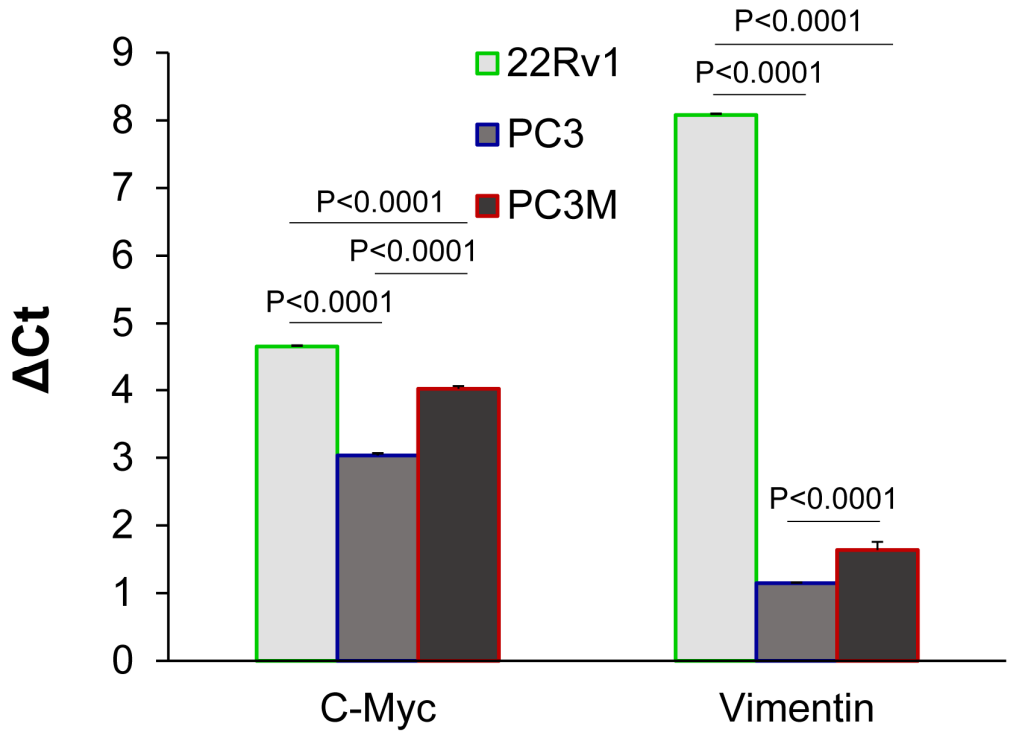
Supplementary Figure 18. Relation between protein expression and number of magnetic beads required for cell capture in each capture zone. (A) Flow cytometry histogram and (**B**) median fluorescence intensity of vimentin expression in PC3 cells subsequent to cell sorting within the microfluidic device. Prior to analysis, the cells were fixed with 4% PFA, permeabilized with 0.3% TX-100, and incubated with a specific antibody modified with ssDNAs that were subsequently hybridized with CP1 and CP2. The cells were sorted using a microfluidic device in which the 8 capture zones were connected via tubing to facilitate cell release independently from each capture zone. After washing, the cells were released and incubated with vimentin specific antibody for 30 min at room temperature. After washing, the cells were incubated FITC-labelled rabbit antibody for 30 min at room temperature. A Control experiment was carried out using an isotype antibody. (**C**) Determination of the minimum number of magnetic beads required for cell capture at each capture zone using COMSOL simulation and mathematical calculations provided in the Materials and Methods and Table S1. (**D**) Plot of the minimum number of beads required for cell capture vs. the median fluorescence intensity at each capture zone. Error bars represent the s.d. of technical replicates (N=3).



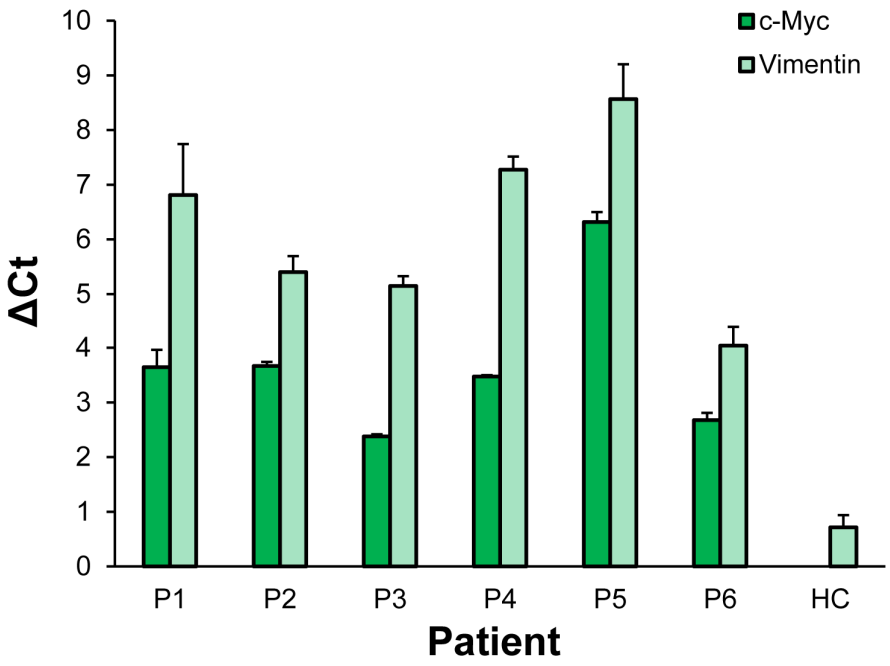
Supplementary Figure 19. RT-qPCR analysis of c-Myc and vimentin mRNAs in CTCs collected from the blood of mice bearing PC3 xenograft. Analysis was carried out after 1, and 3 weeks, subsequent to RBCs removal using the Ficoll method. Error bars represent the s.d. of technical replicates (N=3). ΔCt represents the difference between the Ct value of the target gene and the housekeeping gene GAPDH. Two-way ANOVA tests were used to analyse the data and Holm−Sidak test was used for multiple comparisons with 95% confidence intervals.



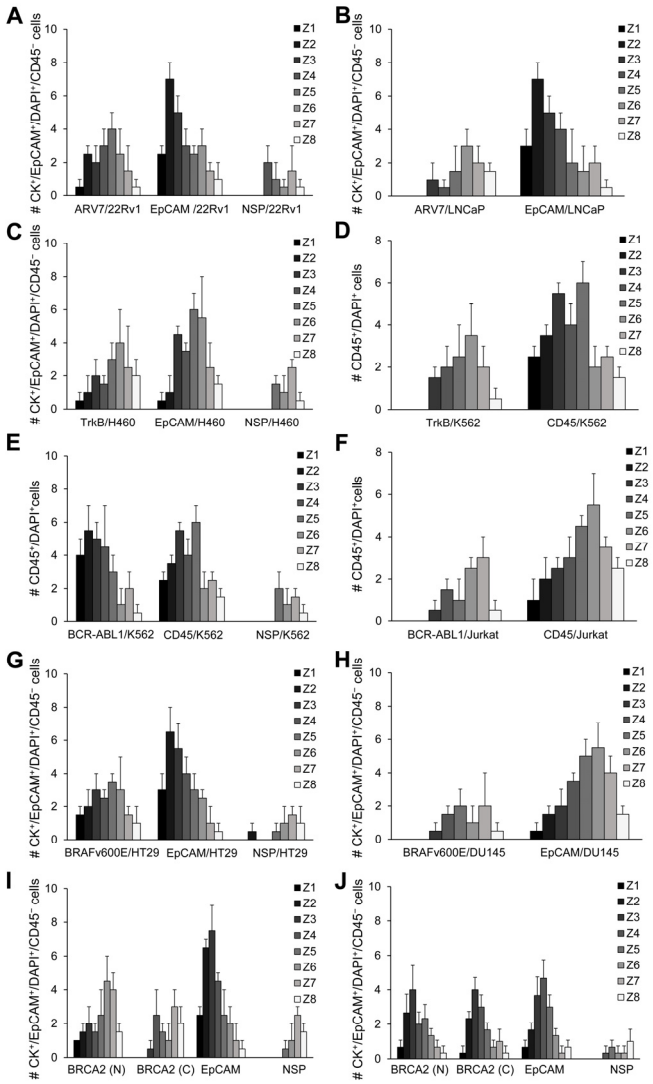
Supplementary Figure 20. RT-qPCR analysis of c-Myc and vimentin mRNAs in CTCs collected from the blood of mice bearing PC3M xenograft. Analysis was carried out after 1 and 3 weeks, subsequent to RBCs removal using the Ficoll method. Error bars represent the s.d. of technical replicates (N=3). ΔCt represents the difference between the Ct value of the target gene and the housekeeping gene GAPDH. A high ΔCt value indicates a low gene expression. Two-way ANOVA tests were used to analyse the data and Holm−Sidak test was used for multiple comparisons with 95% confidence intervals.



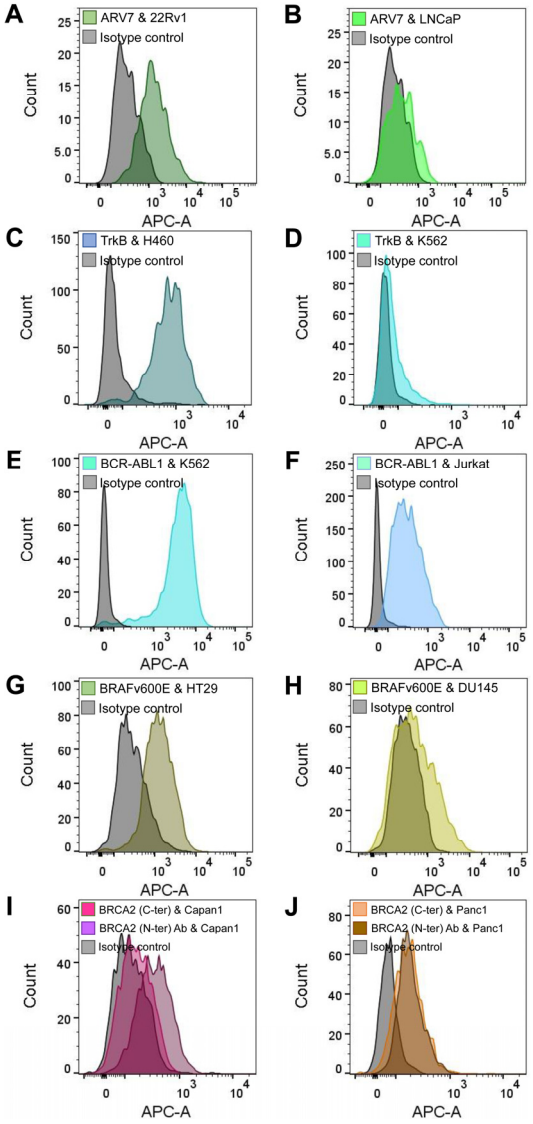
Supplementary Figure 21. RT-qPCR analysis of c-Myc and vimentin mRNAs in 22Rv1, PC3, and PC3M cell lines. ΔCt represents the difference between the Ct value of the target gene and the housekeeping gene GAPDH. Error bars represent the s.d. of technical replicates (N=3). Two-way ANOVA tests were used to analyse the data and Turkey's test was used for multiple comparisons with 95% confidence intervals.



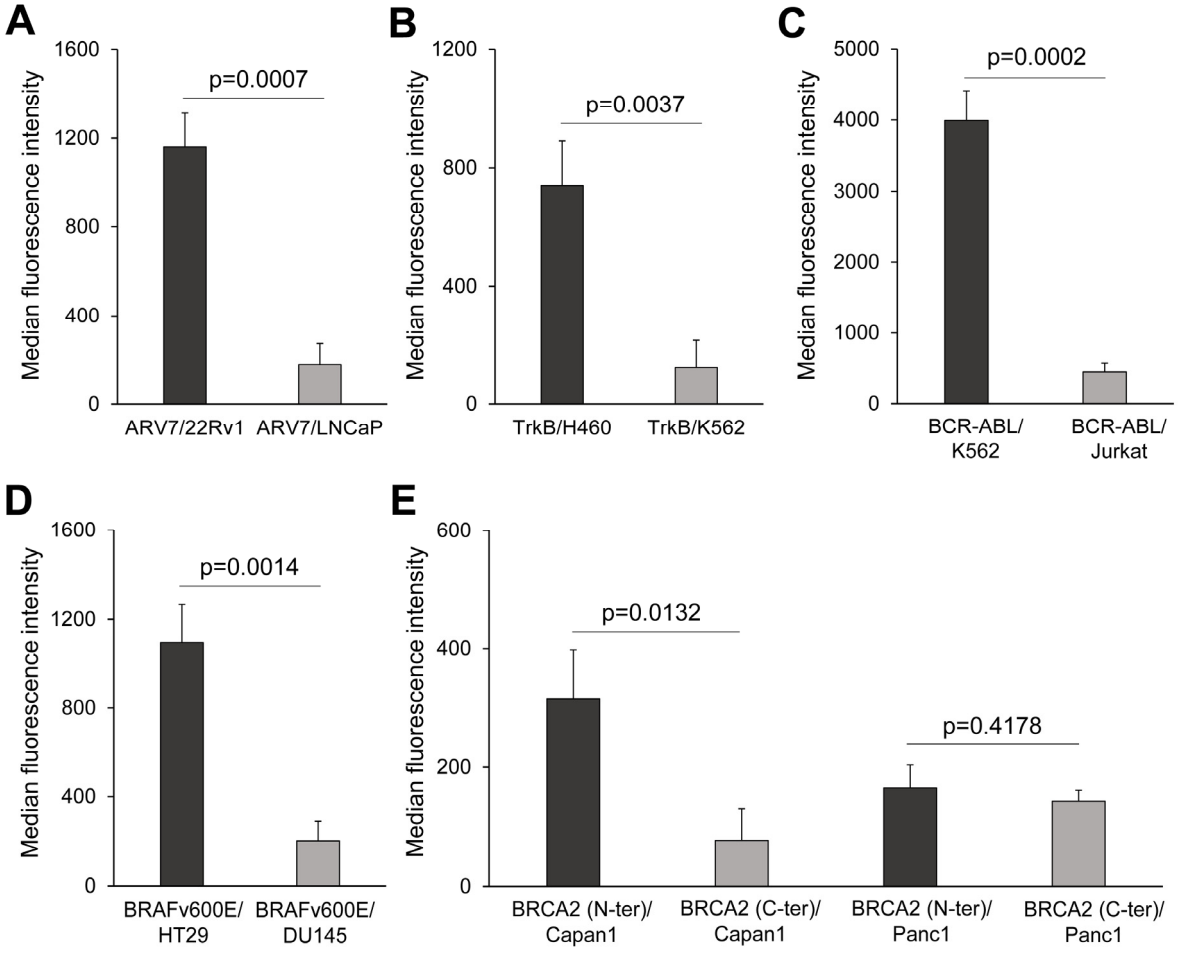
Supplementary Figure 22. RT-qPCR analysis of c-Myc and vimentin mRNAs in CTCs isolated from clinical specimens. Blood samples were collected from a cohort of 6 castration-resistant prostate cancer patients and 1 healthy control and analysis was carried out subsequent to RBCs removal using the Ficoll method. Error bars represent the s.e.m. of technical replicates (N=3). ΔCt represents the difference between the Ct value of the target gene and the housekeeping gene GAPDH.



Supplementary Figure 23. Raw data for the microfluidic analysis of intracellular genetically-altered oncoproteins tested in Figure 5. Five proteins were analysed, including: ARV7 in (**A**) 22Rv1 and (**B**) LNCaP cells, TrkB in (**C**) H460 and (**D**) K562 cells, BCR-ABL1 in (**E**) K562 and (**F**) Jurkat cells, BRAFv600E in (**G**) HT29 and (**H**) DU145, and BRCA2 in (**I**) Capan1 and (**J**) Panc1 cells. A total of 30 cells of 22Rv1, LNCaP, HT29, DU145, Capan1, and Panc1 were spiked in 1 mL of healthy blood. A total of 30 cells of H460, K562 and Jurkat were suspended in PBS. Prior to analysis, RBCs were removed using the Ficoll method. Cells were fixed with 4% PFA, permeabilized with 0.3% TX-100, and incubated with a specific antibody modified with ssDNAs that were subsequently hybridized with MNPs-labelled CP1 and MNPs-labelled CP2. A Control experiment was carried out in which nonspecific probe (NSP) was used instead of CP1 and CP2. Another experiment was carried out in which the cells were captured by targeting either EpCAM or CD45. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1} and stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. Error bars represent the s.e.m. of biological replicates (N=3).

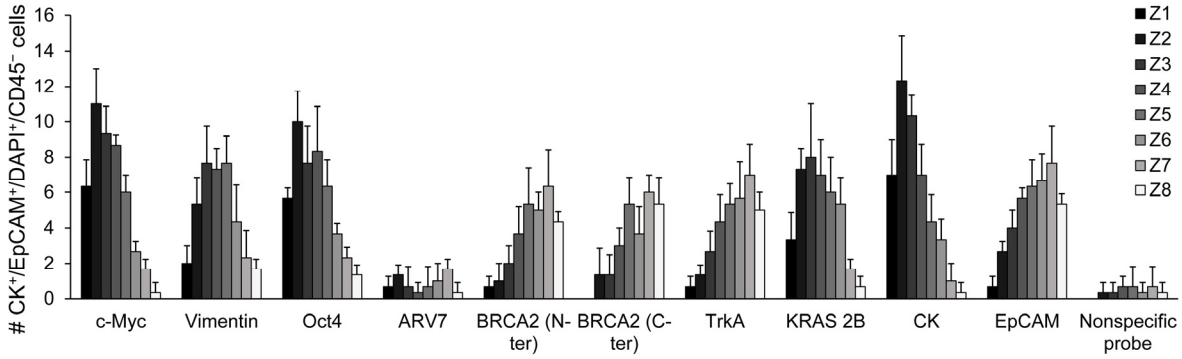

A
ARV7 & 22Rv1
Isotype control
B
ARV7 & LNCaP
Isotype control
C
TrkB & H460
Isotype control
D
TrkB & K562
Isotype control
E
BCR-ABL1 & K562
Isotype control
F
BCR-ABL1 & Jurkat
Isotype control
G
BRAFv600E & HT29
Isotype control
H
BRAFv600E & DU145
Isotype control
I
BRCA2 (C-ter) & Capan1
BRCA2 (N-ter) Ab & Capan1
Isotype control
J
BRCA2 (C-ter) & Panc1
BRCA2 (N-ter) Ab & Panc1
Isotype control
Count
APC-A

Supplementary Figure 24. Flow cytometric analysis of intracellular genetically-altered oncoproteins. Analysis of ARV7 in (**A**) 22Rv1 and (**B**) LNCaP cells, TrkB in (**C**) H460 and (**D**) K562 cells, BCR-ABL1 in (**E**) K562 and (**F**) Jurkat cells, BRAFv600E in (**G**) HT29 and (**H**) DU145 cells, and BRCA2 in (**I**) Capan1 and (**J**) Panc1 cells. Prior to analysis, cells were fixed with 4% PFA, permeabilized with 0.2% TX-100, and incubated with the specific antibody for 30 min at room temperature. Control experiments were carried out in which the cells were incubated with rabbit IgG isotype control for 30 min at room temperature. After washing, the cells were incubated with APC-labelled anti-rabbit IgG for 30 min at room temperature. For BCR-ABL1 analysis, the cells were incubated with APC-labelled anti-mouse IgG for 30 min at room temperature. Control experiments were carried out in which the cells were incubated with mouse IgG isotype control for 30 min at room temperature. After washing, the cells were incubated with 10 µL of APC-labelled anti-mouse IgG for 30 min at room temperature.

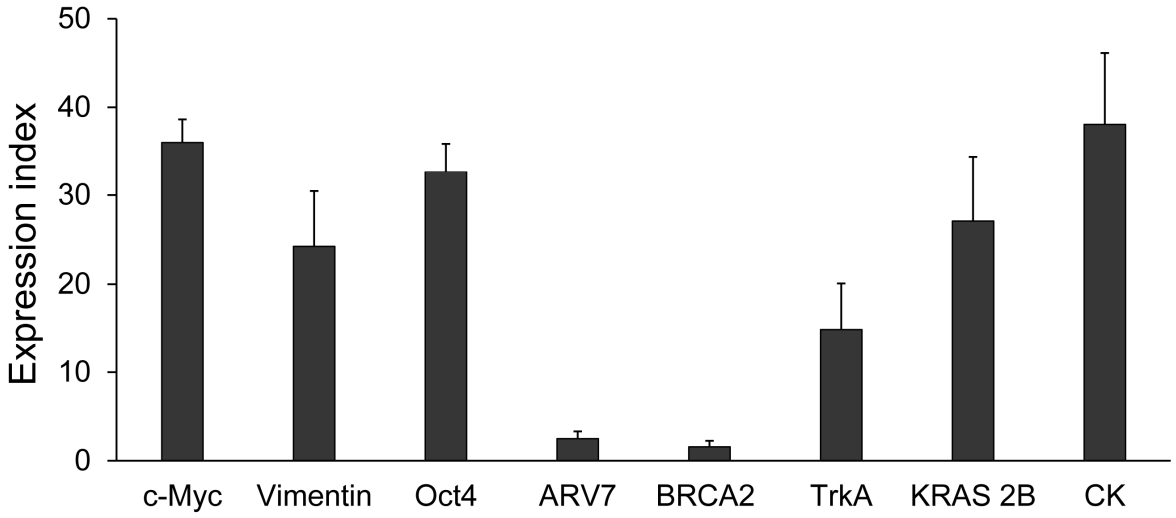


Supplementary Figure 25. Median fluorescence intensities determined from flow cytometric analysis of intracellular genetically-altered oncoproteins. Analysis of (**A**) ARV7 in 22Rv1 LNCaP cell, (**B**) TrkB in H460 and K562 cells, (**C**) BCR-ABL1 in K562 and Jurkat cell, (**D**) BRAFv600E in HT29 and DU145 cells, and (**E**) BRCA2 in Capan1 and MDA-MB-231 cells. Prior to analysis, cells were fixed with 4% PFA, permeabilized with 0.2% TX-100, and incubated with the specific antibody for 30 min at room temperature. Control experiments were carried out in which the cells were incubated with rabbit IgG isotype control for 30 min at room temperature. After washing, the cells were incubated with APC-labelled anti-rabbit IgG for 30 min at room temperature. For BCR-ABL1 analysis, the cells were incubated with APC-labelled anti-mouse IgG for 30 min at room temperature. Control experiments were carried out in which the cells were incubated with mouse IgG isotype

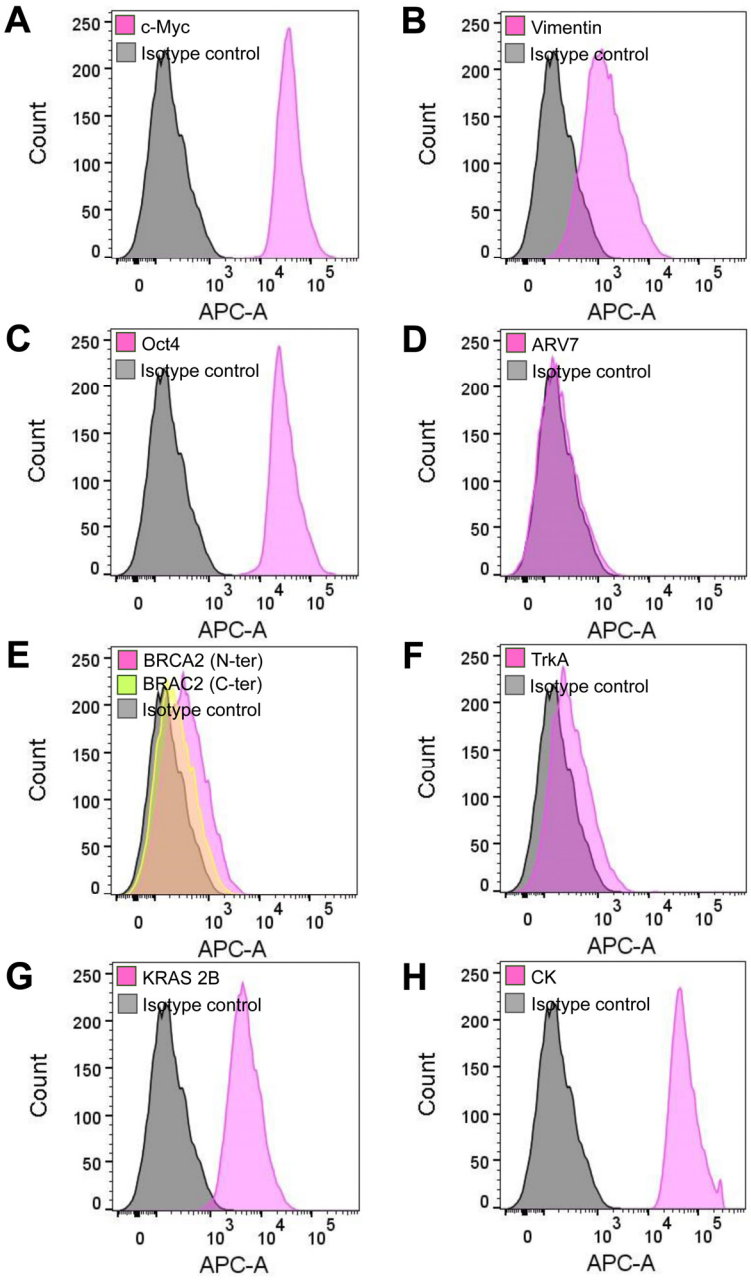
control for 30 min at room temperature. After washing, the cells were incubated with 10 µL of APC-labelled anti-mouse IgG for 30 min at room temperature. Error bars represent the s.d. of biological replicates (N=3). Unpaired two-tailed Student's *t*-test was used to the analyse the data.



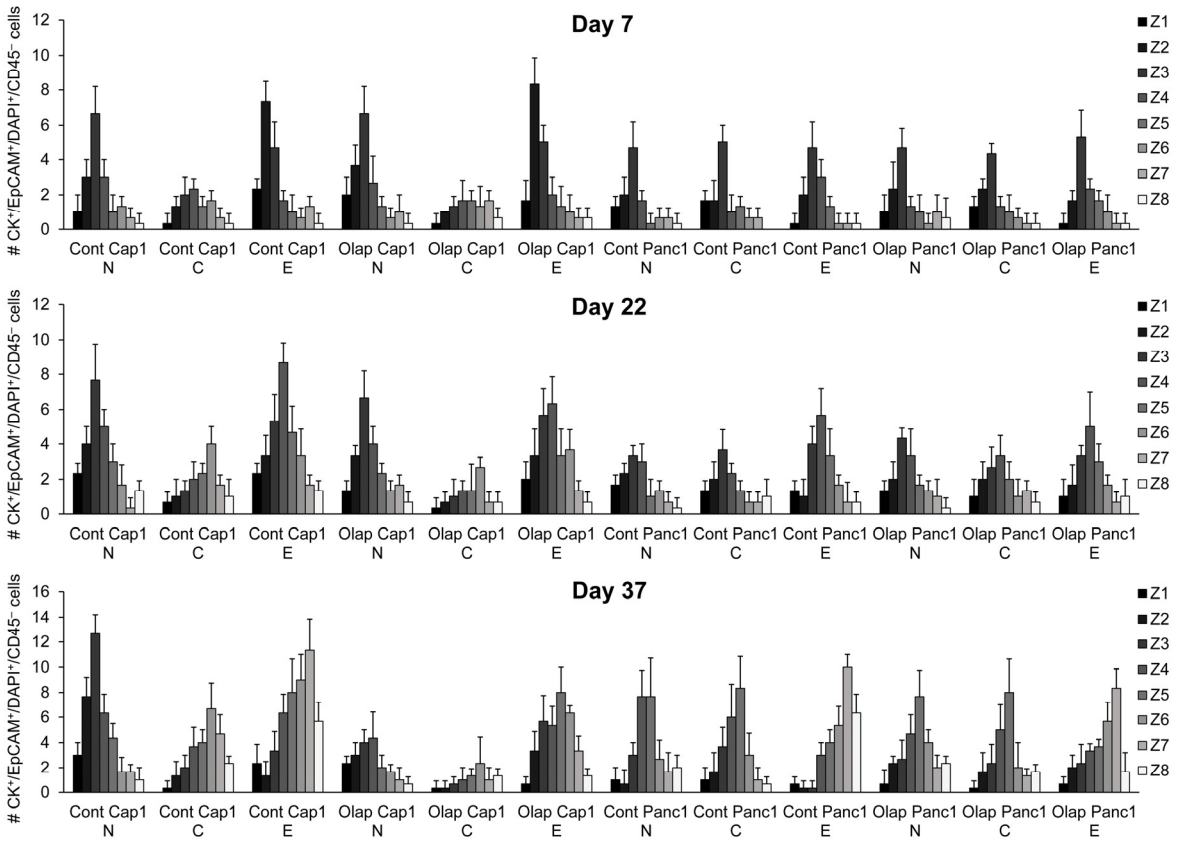
Supplementary Figure 26. Raw data for the parallel analysis of intracellular proteins in MDA-MB-231 cells using the microfluidic approach. Eight proteins were analyzed, including c-Myc, vimentin, Oct4, ARV7, truncated BRCA2, TrkA, KRAS 2B, and CK in healthy blood samples spiked with MDA-MB-231 cells. A total of 50 MDA-MB-231 cells were spiked in 1 mL of healthy blood. Prior to analysis, RBCs were removed using the Ficoll method. Cells were fixed with 4% PFA, permeabilized with 0.3% TX-100, and incubated with a specific antibody modified with ssDNAs that were subsequently hybridized with MNPs-labelled CP1 and MNPs-labelled CP2. Another experiment was carried out in which the cells were captured by MNPs-labelled EpCAM antibody. A control experiment was carried out in which the cells were incubated with c-Myc specific antibody modified with ssDNAs then subsequently incubated with nonspecific probes modified with MNPs. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1} and stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. Error bars represent the s.d. of biological replicates (N=3).



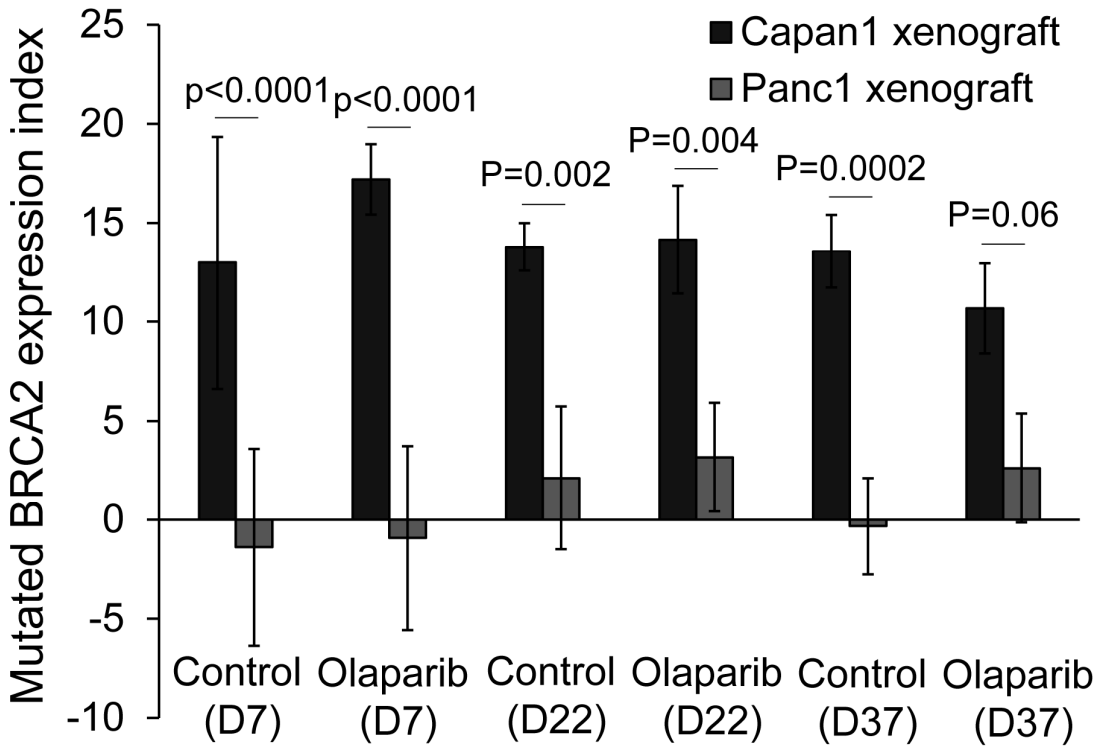
Supplementary Figure 27. Expression levels of intracellular proteins analysed in parallel using the microfluidic approach. Eight proteins were analysed, including c-Myc, vimentin, Oct4, ARV7, truncated BRCA2, TrkA, KRAS 2B, and CK in healthy blood samples spiked with MDA-MB-231 cells. A total of 50 MDA-MB-231 cells were spiked in 1 mL of healthy blood. Prior to analysis, RBCs were removed using the Ficoll method. Cells were fixed with 4% PFA, permeabilized with 0.3% TX-100, and incubated with a specific antibody modified with ssDNAs that were subsequently hybridized with MNPs-labelled CP1 and MNPs-labelled CP2. Another experiment was carried out in which the cells were captured by targeting EpCAM. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1} and stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^{+} and/or $EpCAM^{+}/DAPI^{+}/CD45^{-}$ cells were counted. Error bars represent the s.d. of biological replicates (N=3).



Supplementary Figure 28. Flow cytometric analysis of several intracellular proteins in MDA-MB-231. Analysis of (**A**) c-Myc, (**B**) vimentin, (**C**) Oct4, (**D**) ARV7, (**E**) BRCA2, (**F**) TrkA, (**G**) KRAS 2B, and (**H**) CK in MDA-MB-231 cells. Prior to analysis, cells were fixed with 4% PFA, permeabilized with 0.2% TX-100, and incubated with the specific antibody for 30 min at room temperature. Control experiments were carried out in which the cells were incubated with rabbit IgG isotype control for 30 min at room temperature. After washing, the cells were incubated with APC-labelled anti-rabbit IgG for 30 min at room temperature. After washing, the cells were incubated with 10 µL of APC-labelled anti-mouse IgG for 30 min at room temperature.



Supplementary Figure 29. Raw data for the microfluidic analysis of truncated BRCA2 protein in pancreatic tumour xenografts tested in Figure 5. Tumour xenografts were generated by injecting either Capan1 or Panc1 cells into female athymic nude mice. After tumour formation at day 7, the mice were randomly divided into control and treated groups (n=3). Mice in the treated group received 50 mg/kg olaparib every other day for 4 weeks. Mice in the control group received only the vehicle. At day 7, 22 and 37, blood samples were collected from both treated and control mice. Prior to analysis, RBCs were removed using the Ficoll method. The Cells were fixed with 4% PFA, permeabilized with 0.3% TX-100. The cells were incubated with either an antibody specific for the N-terminus or C-terminus of BRCA2 protein and modified with ssDNAs that were subsequently hybridized with MNPs-labelled CP1 and MNPs-labelled CP2. Another experiment was carried out in which the cells were captured using MNPs-labelled EpCAM antibody. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1} and stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. Error bars represent the s.d. of biological replicates (N=3).



Supplementary Figure 30. Extended four-week analysis of the expression of truncated BRCA2 protein in pancreatic tumour xenografts tested in Figure 5. Tumour xenografts were generated by injecting either Capan1 or Panc1 cells into female athymic nude mice. After tumour formation at day 7, the mice were randomly divided into control and treated groups (n=3). Mice in the treated group received 50 mg/kg olaparib every other day for 4 weeks. Mice in the control group received only the vehicle. At day 7, 22 and 37, blood samples were collected from both treated and control mice. Prior to analysis, RBCs were removed using the Ficoll method. The Cells were fixed with 4% PFA, permeabilized with 0.3% TX-100. The cells were incubated with either an antibody specific for the N-terminus or C-terminus of BRCA2 protein and modified with ssDNAs that were subsequently hybridized with MNPs-labelled CP1 and MNPs-labelled CP2. Another experiment was carried out in which the cells were captured using MNPs-labelled EpCAM antibody. The cells were loaded into the microfluidic device at a flow rate of 2 mL h^{-1} and stained with APC-labelled anti-CK, APC-labelled anti-EpCAM, AF488-labelled anti-CD45, and DAPI. Only CK^+ and/or $EpCAM^+/DAPI^+/CD45^-$ cells were counted. Error bars represent the s.d. of biological replicates (N=3). Two-way ANOVA tests were used to analyse the data in Figure 5 and Holm−Sidak test was used for multiple comparisons.

REFERENCES

1. Hughes, A. J. et al. Single-cell western blotting. *Nat. Methods* **11**, 749-755 (2014).