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## **Supplementary Information**

## Microfluidic device fabrication

Microfluidic devices were fabricated using the standard photolithography. Patterns for the microfluidic ratchet device is first fabricated on a silicon wafer with two layers. The microstructures patterns were designed using DraftSight (Dassault Systems, France), and translated onto optical photomasks. One high-resolution photomask was used to generate the microscale funnel constrictions while one lowresolution photomask was used for the flow channels. In the first layer, the microstructures in the central sorting region are fabricated using negative photoresist SU-8 8025 (Microchem, MA). The silicon wafer was coated with negative photoresist SU-8 8025, spun at 3000 rpm for 30 sec to produce a 30 µm thick layer. The wafer was then baked at 95 °C for 5 min and exposed to UV light though the optical photomask, followed by post-bake steps at 65 °C (1 min), 95 °C (4 min), and 65 °C (1 min), and washed with isopropanol. The second layer formed the supporting flow channels and aligned with the first pattern. The second set of SU-8 features patterned as described above, with a spin speed of 2200 rpm for 30 sec to create the height of this layer as 40  $\mu$ m. The structures were hardened by ramping the temperature from 40 °C to 165 °C, at increments of 15 °C/10 min. The silicon wafer was then incubated at 165 °C for 30 min and cooled to 65 °C by ramping the temperature by 50 °C/10 min. The final heights of the first and second layer were measured to be 29.6 and 40.1  $\mu$ m, respectively.

## PDMS device fabrication

The replicas of the silicon wafer molds were fabricated using soft-lithography of Sylgard 184 polydimethylsiloxane (PDMS) silicone. The silicon wafer with microstructures was placed on a 15-cm diameter Petri dish, and secured in the center with the tape. PDMS mixture (base:curing agent = 10:1) was poured into the petri dish to a thickness of ~5 mm, and the petri dish was placed in a vacuum

chamber for 15 min to void air bubbles from the features. The petri dish was subsequently baked at 65 °C for 2 hours to cure the PDMS. Cured PDMS was gently peeled off from the silicon wafer, and holes were made using a 0.5 or 6 mm hole punch (Harris, CA). PDMS layer is attached to a glass slide by 90 sec of activation in air plasma (Harrick Plasma, NY), followed by baking the PDMS devices at 65 °C for 10 min. All PDMS devices were cooled to the room temperature before using them.

## **Supplementary Figures**

Supplementary Fig. S1. The PDMS baffle with 6 mm deep reservoirs was manufactured using a stereolithography (SLA) 3D printed mould and subsequently fabricated using a standard soft lithography technique. The PDMS baffle was carefully overlaid onto a PEN membrane slide without creating wrinkles on a PEN membrane. After the microfluidic enrichment, cell suspensions were introduced into each reservoirs of the PDMS baffle, followed by overlaying the polyethylene glycol diacrylate (PEGDA) solutions for single cell isolation using laser capture microdissection.



Supplementary Fig. S2. Principle of microfluidic ratchet mechanism in forward (A) and backward (B) direction. The principle of the microfluidic ratchet mechanism involves the deformation of single cells through funnel-shaped constrictions where the opening of the constriction is smaller than the diameter of the cell. Force required to deform cells along the direction of the funnel (A) is less than that against the direction of the flow (B).



Supplementary Fig. S3. A) All samples including WGA products from single LNCaP cells (L1-L5) and a piece of PEGDA (P1-P3) showed the strong smear using the agarose gel electrophoresis. The smears on negative controls result from non-target amplification due to the high processivity Phi29 DNA polymerase used in the REPLI-g WGA protocol. B) qPCR assay targeting ACTB was used to check the quality of DNA prior to sequencing. All PEGDA samples showed high Ct values (>30), indicating no target genes were amplified.



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• L1-L5: WGA products from LNCaP cells

 P1-P3: WGA products from a piece of PEGDA



Supplementary Fig. S4. Review software for CTC enumeration. This software analyzes the images obtained from the Zeiss LSM 780 system to interpret spectral data and to rank individual events based on the likelihood of being CTCs. When the samples are scanned using the Zeiss LSM 780 system, a gigapixel image cube of 32 different spectral channels is generated. Using the gigapixel spectral image cube and the pre-defined emission peak criteria, the software analyzes the spectral data and ranks cells based on their spectral emission from the most likely to least likely to be a CTC. The software then generates a simple interface for users to manually review the emission spectrum for each individual cell. After the reviewing process, a 'CTC map' is generated, which contains the positional information of each target cells for further single-cell extraction process using LCM.



Supplementary Fig. S5. Single cell analysis validation using targeted next generation sequencing. Single UM-UC13 cells, single UM-UC3 cells, and gDNA from both cell lines were sequenced. The SNPs that were detected included numerous cell line specific tumor mutations, including KRAS, RB1, and TP53.

