Supplementary Information

## Size-Tunable Microvortex Capture of Rare Cells

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Aspect Ratio	Length (L)	Width (W)	Height (H)	Channel Expansion Ratio
AR = L/W	(µm)	(µm)	(µm)	$ER = W/W_{ch}$
1	930	930	70	23.25
2	930	465	70	11.625
3	930	310	70	7.75

Supplementary Figure 1. Microfluidic device geometry and trapping reservoir (cavity) dimensions. Different cavity aspect ratios (AR) are designed to test radial migration of particles in microvortices. Cavity length (L), width (W) and height (H) are along the X, Y and Z directions respectively. Cavity is placed 1 cm away from the inlet. All cavities are made of the same height (H = 70  $\mu$ m). Rectangular channel width (W<sub>ch</sub>) is 40  $\mu$ m and channel height (H<sub>ch</sub>) is 70  $\mu$ m.



**Supplementary Figure 2**. Microvortex core characterization. (a) COMSOL Multiphysics model of velocity distribution in microcavity (AR =2) recirculation region. Different vortex topologies generated at different inertial conditions defined by the mean-flow Reynolds number (Re) in a rectangular channel. Vortex core was identified by the local velocity minimum inside the cavity at different flow rates. (b) Schematic illustration of x-y plane of the cavity and location of the vortex core ( $x_0$ ,  $y_0$ ). (c) Vortex core coordinates along x-axis in the cavity at Reynolds number (Re) 100, 150, 200, 250 and 300.



Supplementary Figure 3. Scatter plot of captured cells size distribution and their orbit trajectories in AR = 1 cavity. Cell motion and progression in microvortices illustrates size selective orbit transition in increasing flow conditions at Reynolds number (Re) 100, 125, 150, 175, 200, 225, 250, 275 and 300.



Supplementary Figure 4. Microvortex loading capacity of MCF-7 ( $\lambda > 0.5$ ) cells) and MDA-MB-231 ( $\lambda < 0.5$ ). Flow of concentrated cell suspension for five minutes followed by PBS wash for five minutes in three inertial conditions at Re = 150, 240 and 300. Microvortex capture of MCF-7 with mean cell diameter = 25 µm and MDA-MB-231 = 18 µm show size-selective capacity in phase I (Re = 150) and phase III (Re = 275) respectively.



Bright field (left) and fluorescent (right) images of PBS and 20x diluted blood spiked with MDA-GFP cell lines in Supplementary Figure 5. Captured MDA-GFP orbit trajectories in microvortex flow with PBS and blood. microvortex flow show consistent orbit trajectories. Fluorescently labeled cancer cell line MDA-GFP orbit trajectories observed in phase I (Re = 125), phase II (Re = 200) and phase III (Re = 300).



**Supplementary Figure 6**. Device performance of rare cells capture from blood. (a) Immunofluorescent staining and classification criteria. Collected cells were classified according to immunostains against GFP (green) and CD45 (red), as well as DNA stained with DAPI (blue). MDA-GFP cells are identified as GFP+/CD45-/DAPI+ and WBCs as GFP-/CD45+/DAPI+. (b) Purity and capturing unit efficiency of collected MDA-GFP spiked in blood. (c) Cells released from microvortex flow are able to grow and proliferate after 4 days.



Supplementary Figure 7. Vortex HT device capture efficiency and size selectivity for MDA-GFP in blood. Isolation and capture of MDA-GFP cells spiked in 20X diluted blood were tested on a High-Throughput vortex device under phase I and phase III flow conditions. (a) The captured MDA-GFP population were segmented to large cells with diameter (a > 25  $\mu$ m,  $\lambda$  > 0.5) and small cells (a < 25  $\mu$ m,  $\lambda$  > 0.5). Phase I shows high capture efficiency for large cells ( $\lambda$  > 0.5) while phase III shows high capture efficiency for small cells ( $\lambda$  < 0.5). (b) Size distribution of captured cells from phase I and III, shows a consistent size selectivity for each flow condition with the Vortex HT device.

**Supplementary Movie 1**. Radial migration of two beads motion in confined microcavity (AR) =1 under different flow conditions (Re).

**Supplementary Movie 2**. Radial migration of multiple beads motion in confined microcavity (AR) =1 under different flow conditions (Re).

**Supplementary Movie 3**. Radial migration of MDA-MB-231 cells in different cavity aspect ratios (AR) = 1, 2 and 3. The video illustrates a consistent size-based radial migration across phase I (Re = 100), II (Re = 200) and III (Re = 300) in different cavity geometries.

**Supplementary Movie 4**. Radial migration of MDA-MB-231 cells motion and progression in AR =1 cavity under different flow conditions (Re).

## Supplementary Note. 1 Normalizing cell capture in micro-cavities

To characterize size-based separation and capturing unit efficiency (%), we pass the same number of cells across the cavity in different flow rates. We can change the concentration or the flow time accordingly of each flow rate to get 1000 cells passing over the cavity. Here is the formula of number of cells passed per min.

Number of passed cells = Concentration (C) [#cells/mL] \* Flow rate (Q) [mL/min]\* Time (t) [min]

Number of passed cells =  $\frac{\text{#cells}}{1 \text{ mL}} \times \frac{\text{mL}}{1 \text{ min}} \times \text{min}$