# Supporting Information for

## A microfluidic Platform for Single Cell Fluorometric Granzyme B Profiling

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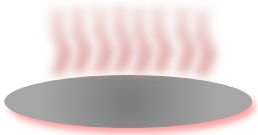
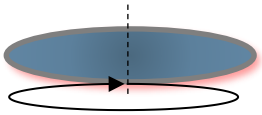
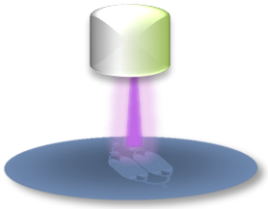
Steps in master mould fabrication



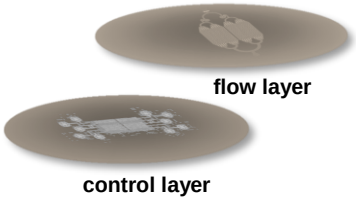
Dehydration of silicon wafer



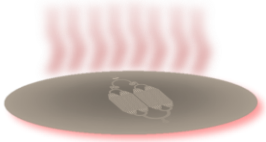
Spin coat SU8 photoresist
Different RPM & photoresist type (SU8 3010/ 3025, MicroChem USA) for different heights of microstructures



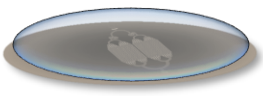
UV-exposure with maskless exposure apparatus
(Nano System Solutions DL-100)




Development
SU8 developer
(MicroChem, Massachusetts, USA)



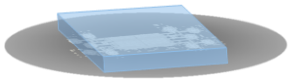
Hard bake for 30 mins at 150 °C



Silanization for 2 minutes
1H,1H,2H,2H-perfluorodecyltrichlorosilane
(Wako Pure Chemical Industries, Ltd.)

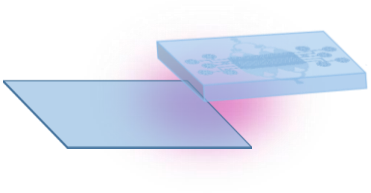
Steps in PDMS device fabrication

Spin coat about 30 µm PDMS mixture on Flow layer mold
10:1 oligomer and curing agent (Sylgard 184 kit, Dow Corning, USA)



Pour approx. 2 mm PDMS on control layer mold
5:1 oligomer and curing agent (Sylgard 184 kit, Dow Corning)

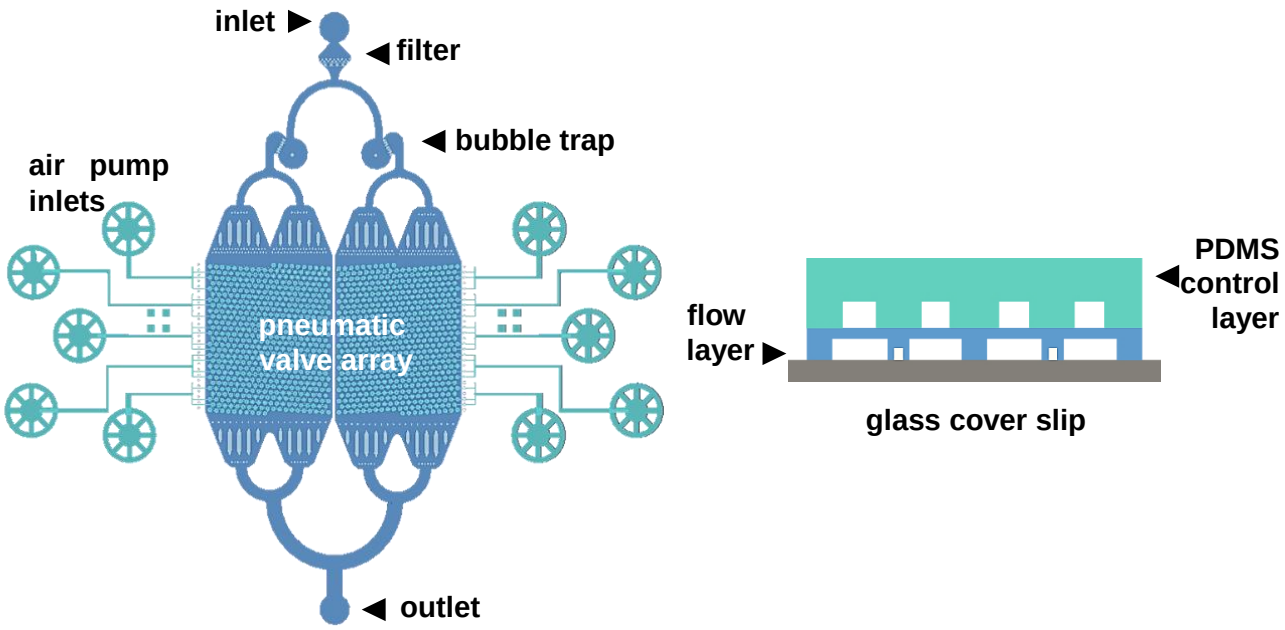
Cut and align the two layers



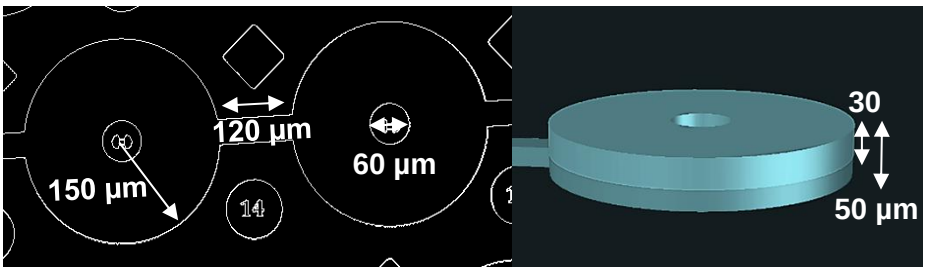
Peel off and expose both PDMS and coverslip to oxygen plasma dry cleaner (Yamato PDC210) for 20 s

Bond PDMS to glass coverslip

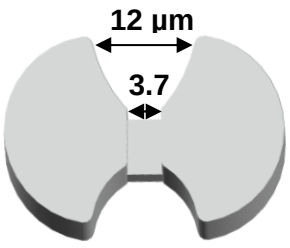
**Figure S1**. Fabrication of the master mold by soft-lithography of SU-8 (3010 and 3025) on 4" Si-wafers and of microfluidic PDMS chip from the silicon master molds.



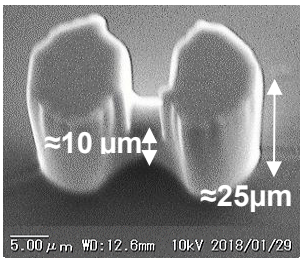

Left, combined CAD design of the control (cyan) and flow layer (blue) moulds. Right, representation of the PDMS chip assembly.




**pneumatic valve dimensions**




**hydrodynamic trap design**




**resulting PDMS trap**

**Figure S2**. Microfluidic chip design and specifications of its main parts. The PDMS device has two layers, a flow layer at the bottom and a control layer on top. The flow layer has microstructures which includes a filter, bubble trap, flow guide pillars, and hydrodynamic traps. The control layer is made up of pneumatic valves, which are cylinder shaped. The actuation of this valves creates a microchamber with the mechanical trap at the center.

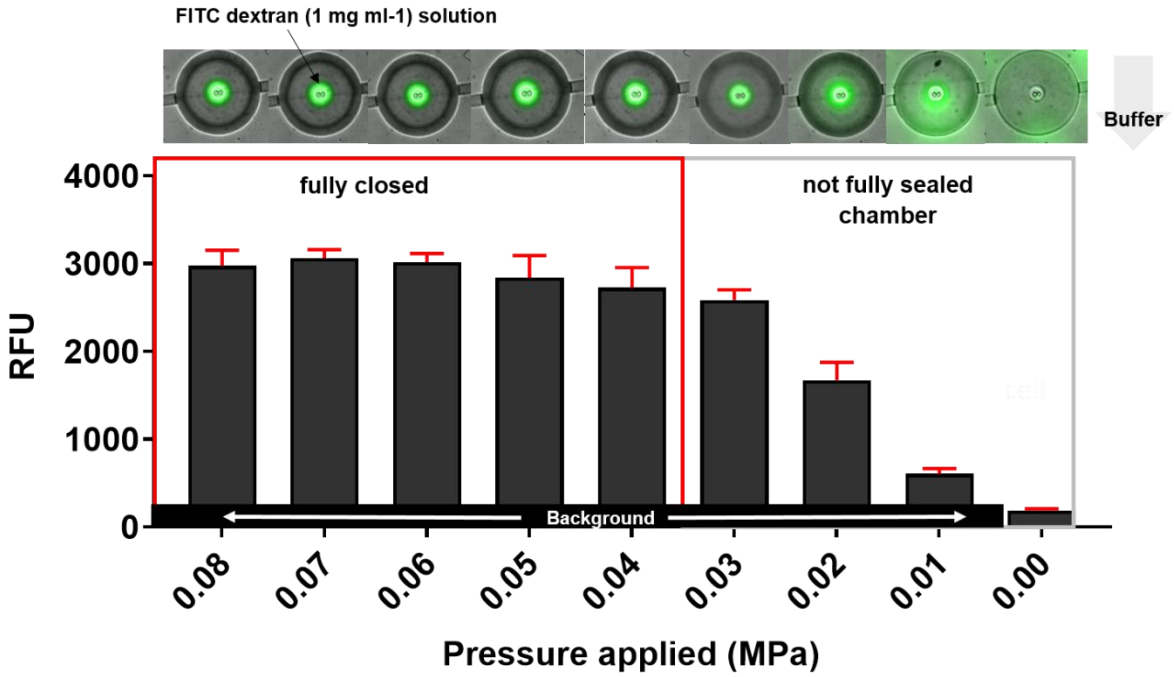



**Figure S3.** To create a sealed microchamber, the pneumatic valves were applied with positive pressure using an air pump (ULVAC DA-60S). The working pressure was determined by actuating the valves at different pressure and measuring the fluorescence of the compartmentalized FITC dextran solution (1 mg $ml^{-1}$, Sigma-Aldrich) inside and outside of the chamber. An applied pressure of 0.07 MPa was later on used in the conduct of the GrB activity assay experiment.

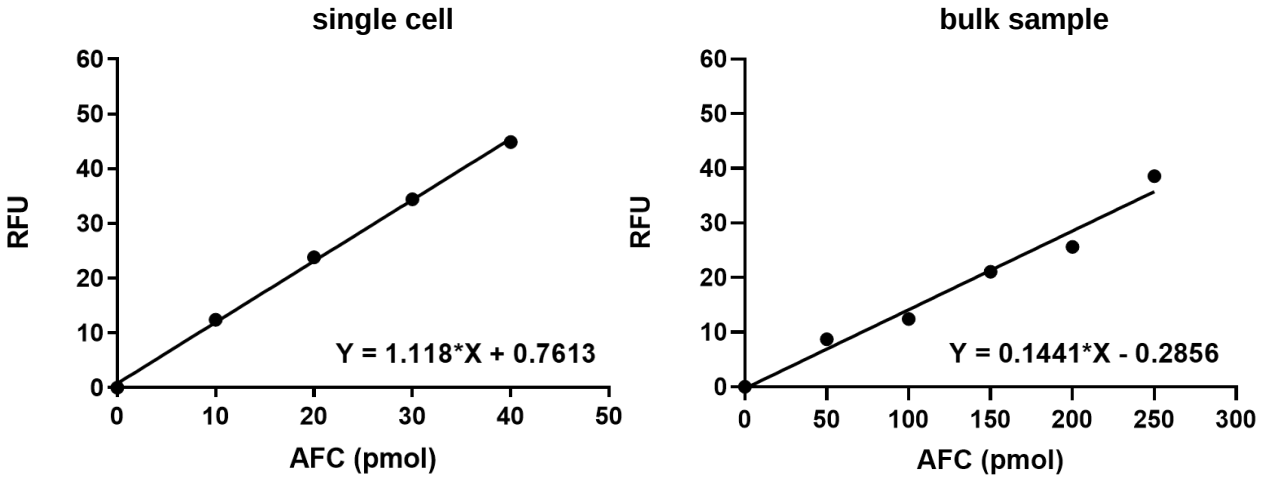
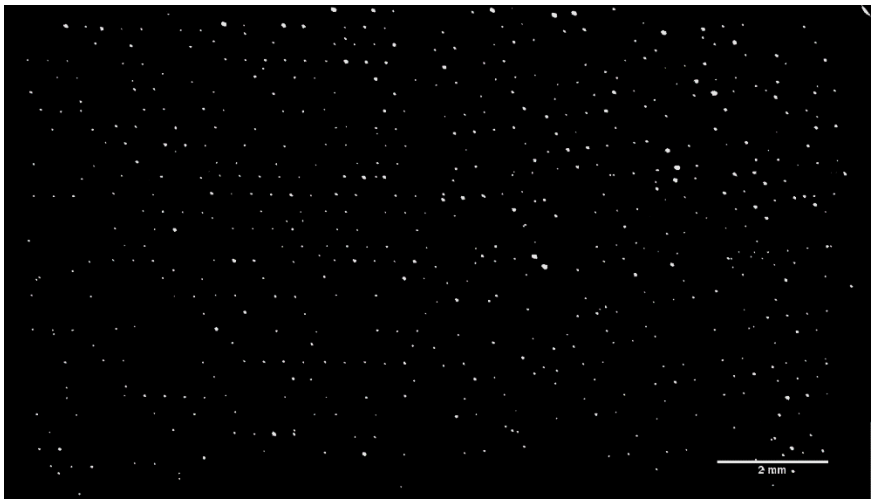
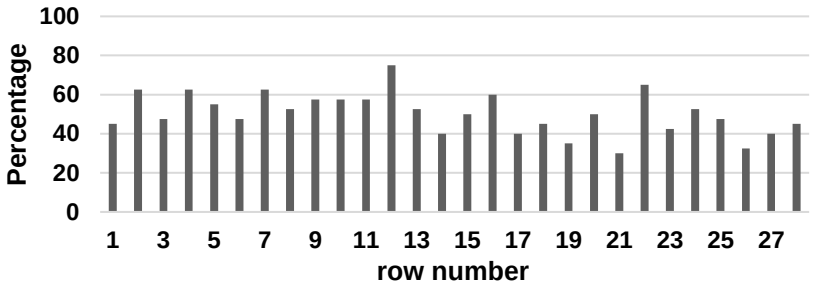


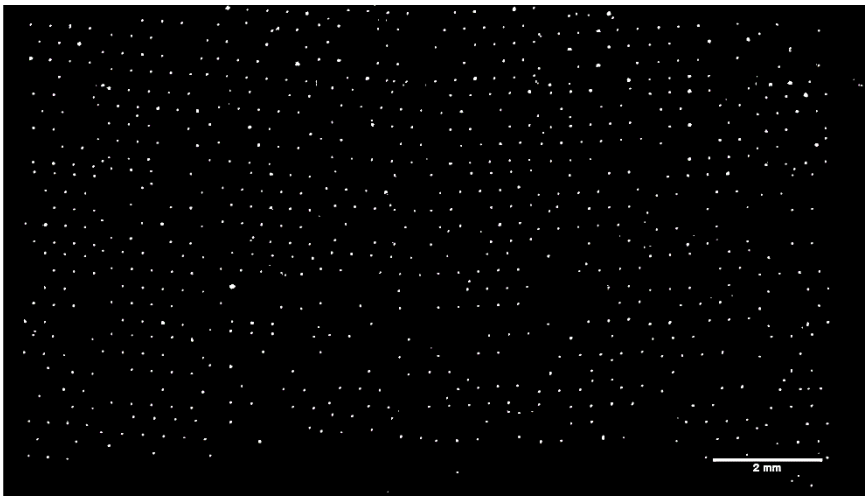

**Figure S4.** Standard curves were taken using the microfluidic device (left) for single cell measurement and a 96-well plate (right) for bulk sample measurement. Different amounts of AFC standard were placed in the microfluidic platform as well as in the well-plate following the assay kit protocol. The fluorescence coming from each of the sampled micro chamber and each well of the 96-well plate was measured. This was used for the interpolation of the GrB activity in the assay experiment.



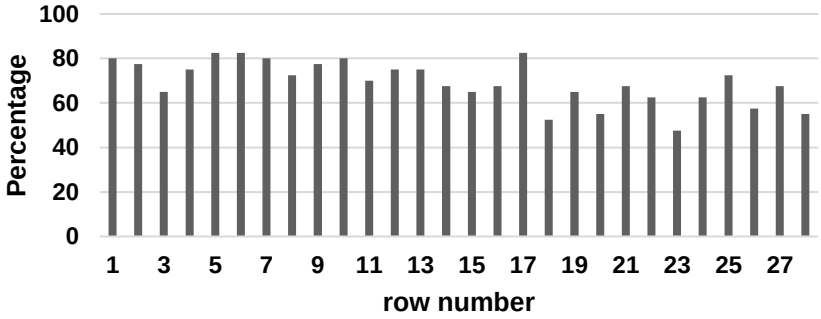

A. Fluorescent samples caught in the hydrodynamic traps at 5 µL/min flow rate



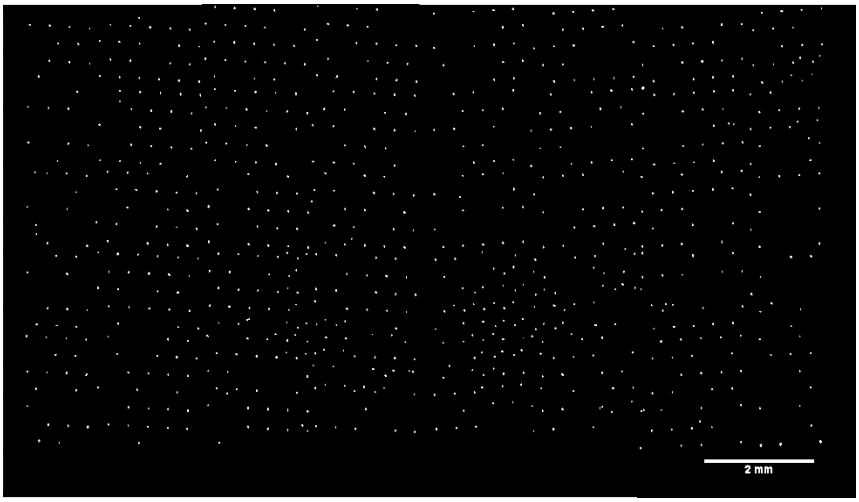

**Percentage of traps occupied for 5µl/min flow rate**



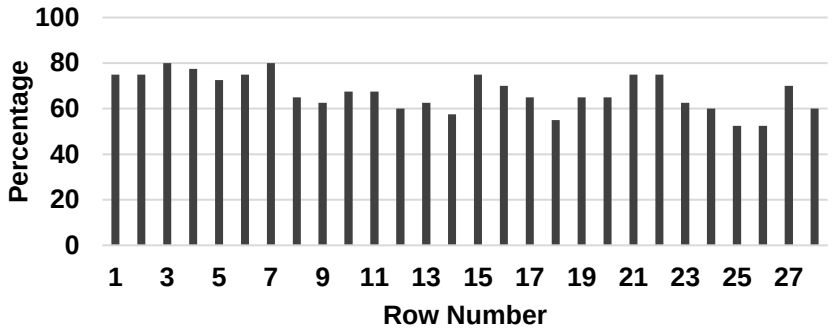

B. Fluorescent samples caught in the hydrodynamic traps at 10 µL/min flow rate

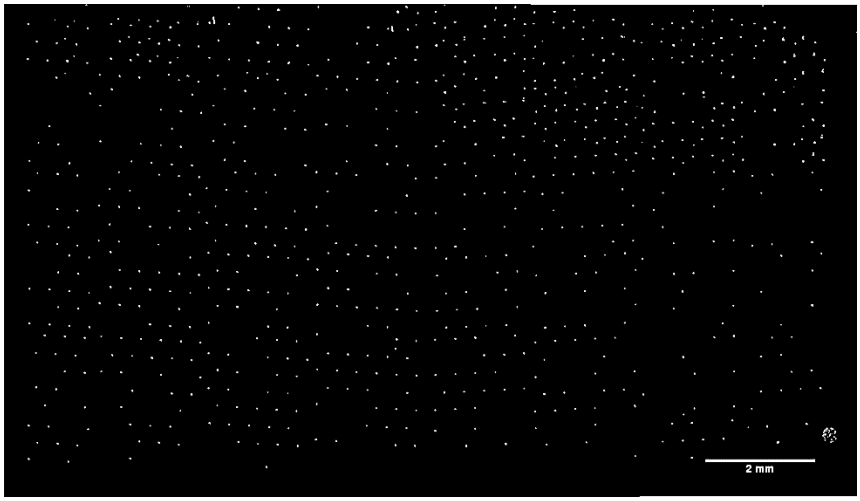



**Percentage of traps occupied for 10 µl/min flow rate**

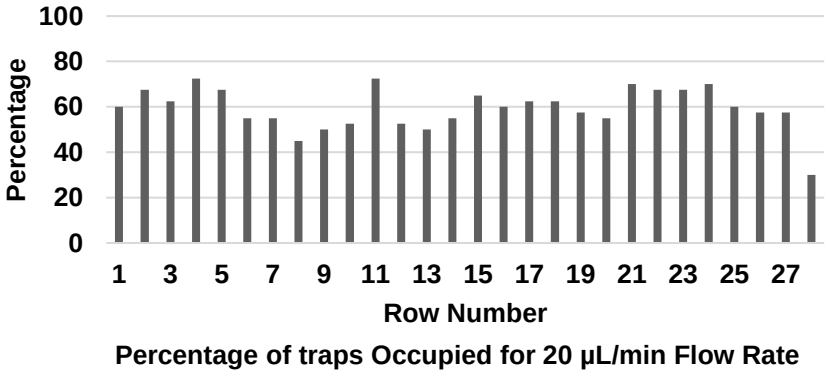


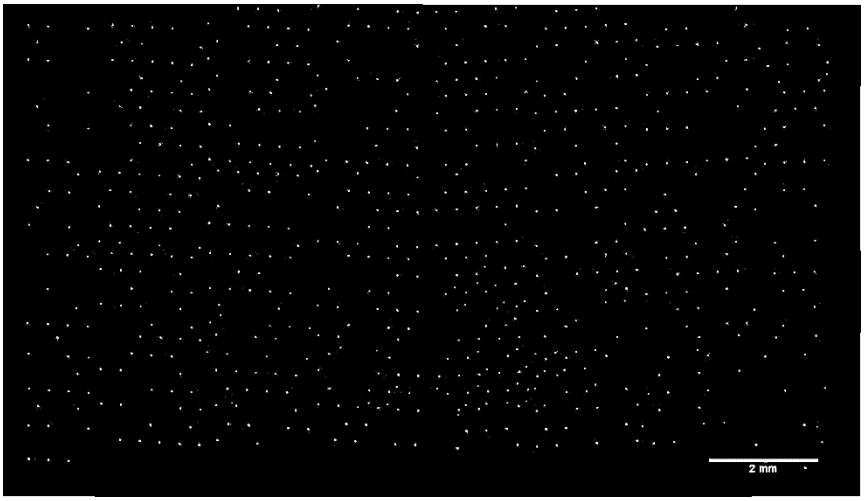

C. Fluorescent samples caught in the hydrodynamic traps at 15 µL/min flow rate



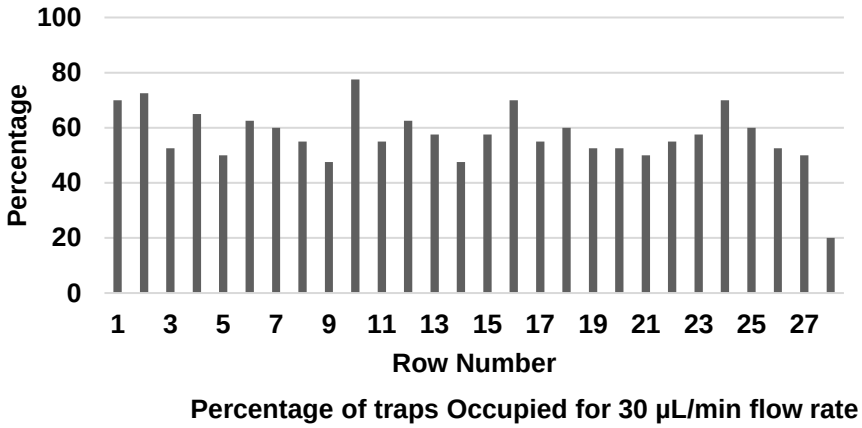



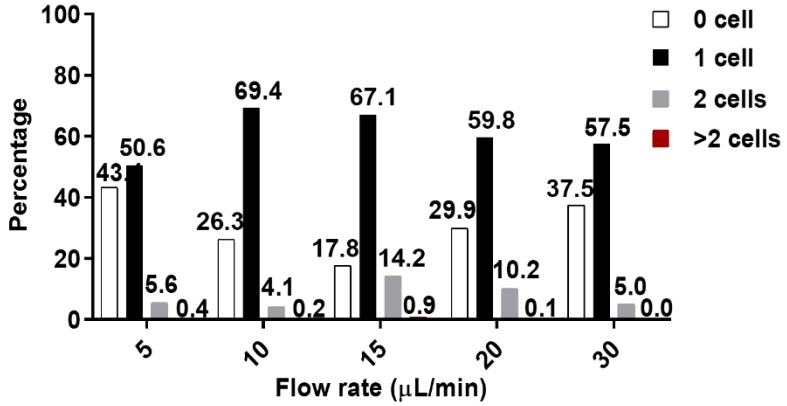

D. Fluorescent samples caught in the hydrodynamic traps at 20 uL/min flow rate






E. Fluorescent samples caught in the hydrodynamic traps at 30 µL/min flow rate

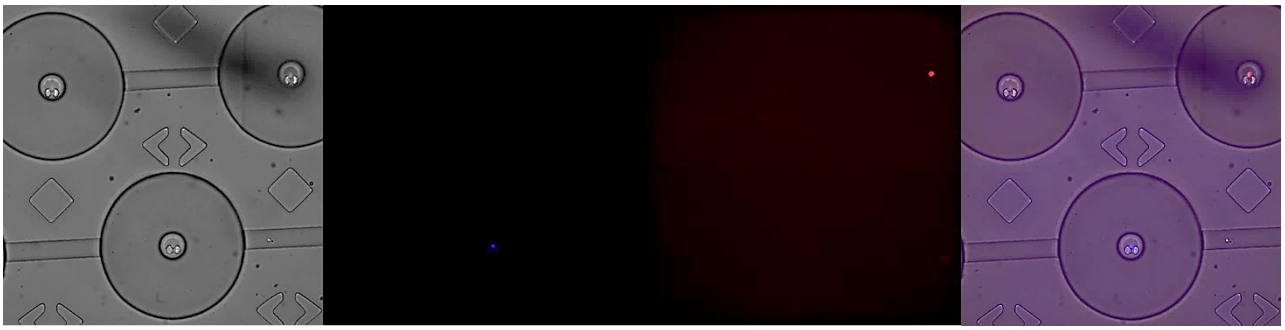



F. Percentage of trap occupancy at different flow rates. The percentage of traps with single capture is shown by the black solid bar, double capture by gray solid bar, while more than 2 cells captured is shown by red bar

**Figure S5.** The microfluidic device was tested for its ability to trap fluorescent cells at different flow rates. Sixty microliters (60 µL) of cell suspension ($10^6$ cells $mL^{-1}$) were made to flow at (A) 5, (B) 10, (C) 15, (D) 20, and (E) 30 µL $min^{-1}$ flow rates. A summary of the percentages of the trap occupancy for single, double, and > 2 cells is shown in F.

A



Bright field | Stain 1 | Stain 2 | Merged

B

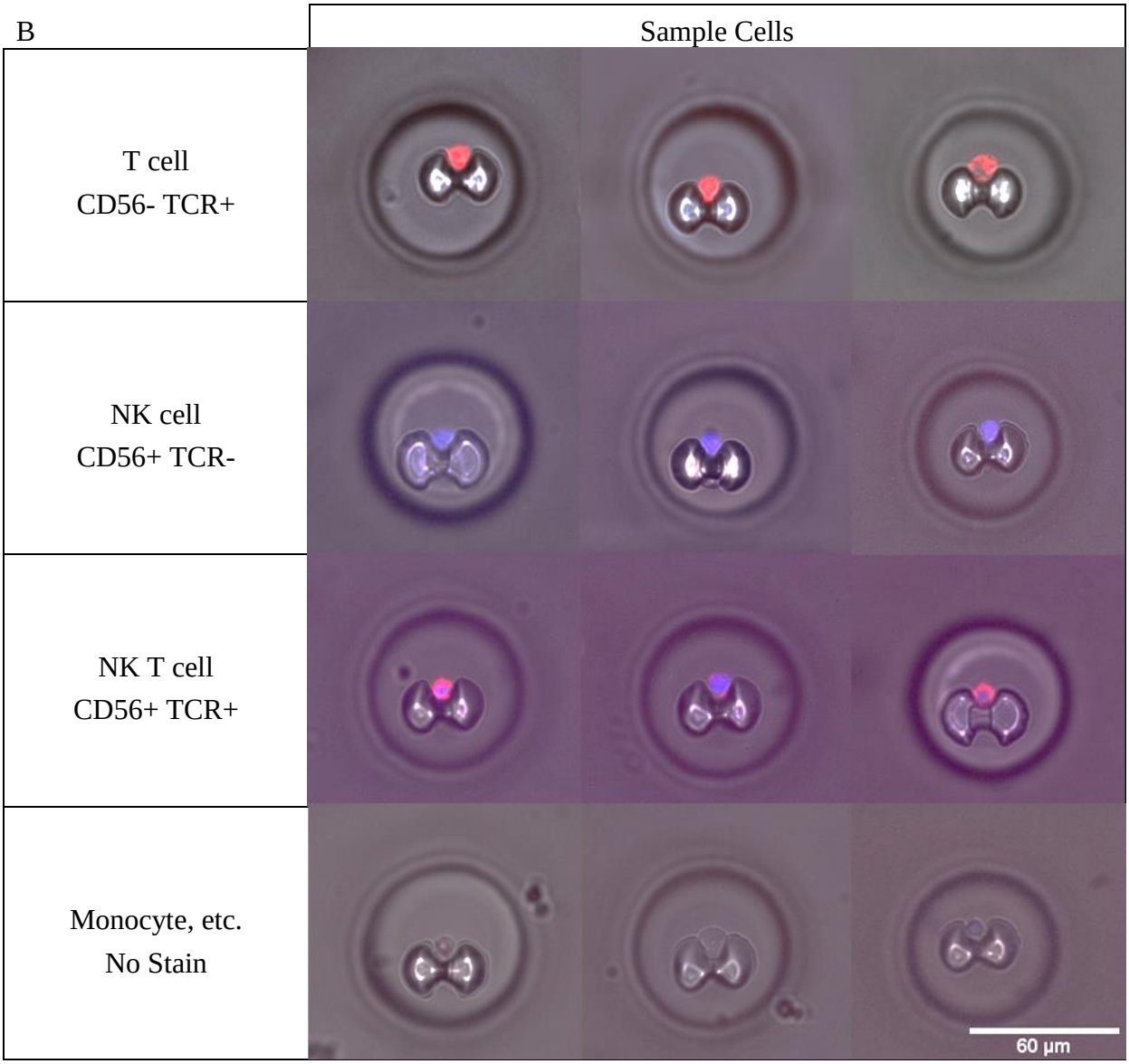



**Figure S6.** Cell surface marker staining was performed on-chip to determine the composition of PBMC samples. FC receptor blocking was first performed before a mixture of antibody markers were introduced into the chip. A) Large view of the same chambers indicating the multiple staining done on the cells. B) Depiction of cell identification utilizing fluorescence measurement of antibody stain.