Title:

Selective Photo-Mechanical Detachment and Retrieval of Divided Sister Cells from Enclosed Microfluidics for Downstream Analyses

Authors and affiliations:

Yu-Chih Chen[1,2,†], Hyoung Won Baac[3,4,†], Kyu-Tae Lee[1], Shamileh Fouladdel[2], Kendall Teichert[5], Jong G. Ok[1,6], Yu-Heng Cheng[1], Patrick N. Ingram[7], A. John Hart[5,8], Ebrahim Azizi[2], L. Jay Guo[1], Max S. Wicha[2], and Euisik Yoon[1,7*]

[1]Department of Electrical Engineering and Computer Science, University of Michigan, 1301 Beal Avenue, Ann Arbor, MI 48109-2122, USA;

[2]University of Michigan Comprehensive Cancer Center, 1500 E. Medical Center Drive, Ann Arbor, MI 48109, USA;

[3]School of Electronic and Electrical Engineering, Sungkyunkwan University, Suwon 440-746, Republic of Korea;

[4]Department of Biomedical Engineering, Sungkyunkwan University, Suwon 440-746, Republic of Korea;

[5]Department of Mechanical Engineering, University of Michigan, 2350 Hayward, Ann Arbor, MI, USA;

[6]Department of Mechanical and Automotive Engineering, Seoul National University of Science and Technology, 232 Gongneung-ro, Nowon-gu, Seoul 01811, Republic of Korea;

[7]Department of Biomedical Engineering, University of Michigan, 2200 Bonisteel, Blvd. Ann Arbor, MI 48109-2099, USA;

[8]Department of Mechanical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue
Cambridge, MA, 02139, USA .

*Corresponding author

†These authors contributed equally to this work.

Yu-Chih Chen

1301 Beal Avenue, Ann Arbor, MI 48109-2122, USA

Tel: 734-272-7113; E-mail: yuchchen@umich.edu.

Euisik Yoon

1301 Beal Avenue, Ann Arbor, MI 48109-2122, USA

Tel: 734-615-4469; E-mail: esyoon@umich.edu.

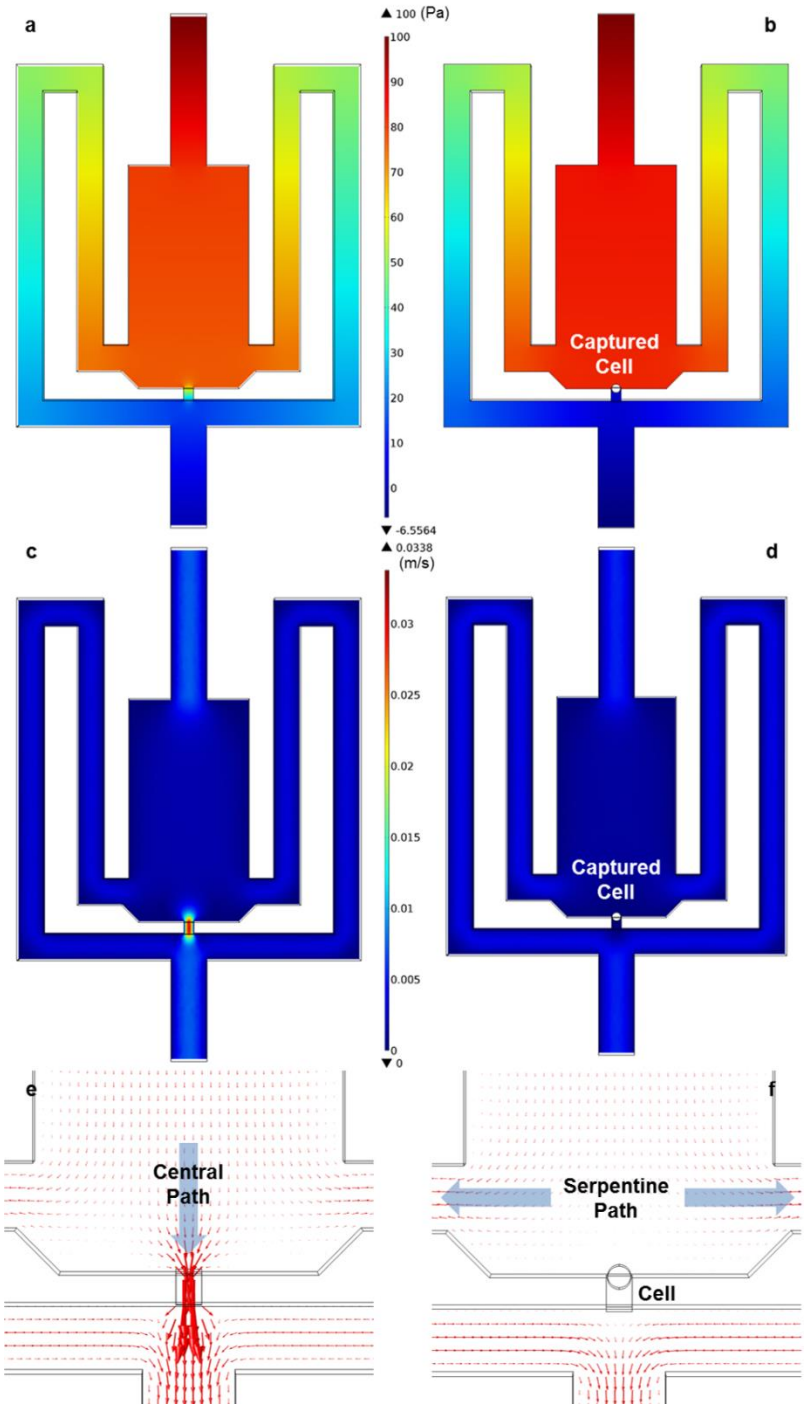
SUPPLEMENTARY INFORMATION

Supplementary Table 1. The 96-gene panel chosen to identify the oncogenic signature of breast cancer cells.

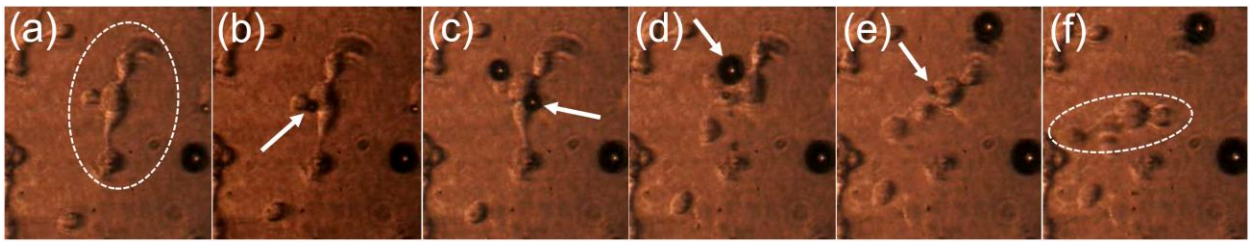
ABCB1	CD146	EZH2	ITGB3	NFKB1	SOX2
ABCG2	CD20	FBXW7	JAG2	NOTCH1	STAP2
AKT1	CD24	GAPDH	KRT18	NOTCH2	TAZ
AKT3	CD3D	GATA3	KRT19	NOTCH3	TGFb1
ALDH1a1	CD44	gp130	KRT5	NUMB	TGFbR1
ALDH1a3	CD45	GSK3B	KRT7	O ct4	TM4SF1
AMOTL2	CDH1	HER2	KRT8	p53	TMEM57
ANXA3	CDH2	HES1	LIN28A	p63	TNKS1BP1
AR	CDH3	HEY2	MCL1	PCNA	TSPAN6
BAX	CTNNB1	HPRT1	MET	PGR	Twist1
BCL2	CXCR1	ID1	MKI67	PI3K	UXT
BRCA1	CXCR4	ID2	MMP9	PTEN	Vimentin
CCND1	DLL1	IL6	MTOR	RAB7A	WNT2
CD11B	EGFR	IL6R	MUC1	SLUG	YAP1
CD133	EpCAM	IL8	NANOG	SNAI1	ZEB1
CD14	ESR1	ITGA6	NESTIN	SOCS3	ZEB2

Supplementary Table 2. 4 types of genes categorized by the differences between asymmetrically and symmetrically divided daughter cells.

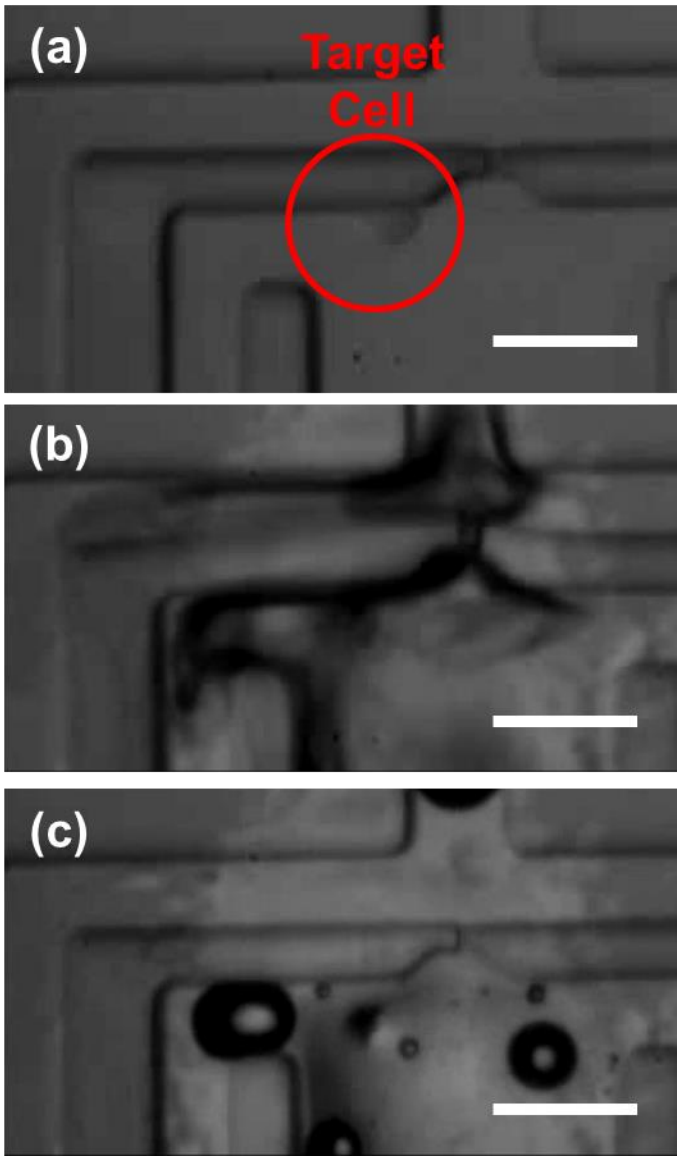
Type I	Type I	Type I	Type II	Type III	Type IV
ABCB1	GATA3	NUMB	BRCA1	ANXA3	ALDH1a1
ABCG2	gp130	O ct4	CD146	BCL2	CXCR1
AKT1	GSK3B	p53	CD3D	TAZ	
AKT3	HPRT1	p63	EZH2	Twist1	
ALDH1a3	ID1	PCNA	HER2		
AMOTL2	ID2	PGR	HES1		
AR	IL6	PI3K	HEY2		
BAX	IL6R	PTEN	ITGB3		
CCND1	IL8	RAB7A	KRT18		
CD11B	ITGA6	SLUG	KRT5		
CD133	JAG2	SNAI1	TMEM57		
CD14	KRT19	SOCS3			
CD20	KRT7	SOX2			
CD24	KRT8	STAP2			
CD44	LIN28A	TGFb1			
CD45	MCL1	TGFbR1			
CDH1	MET	TM4SF1			
CDH2	MKI67	TNKS1BP1			
CDH3	MMP9	TSPAN6			
CTNNB1	MTOR	UXT			
CXCR4	MUC1	Vimentin			
DLL1	NANOG	WNT2			
EGFR	NESTIN	YAP1			
EpCAM	NFKB1	ZEB1			
ESR1	NOTCH1	ZEB2			
FBXW7	NOTCH2				
GAPDH	NOTCH3				



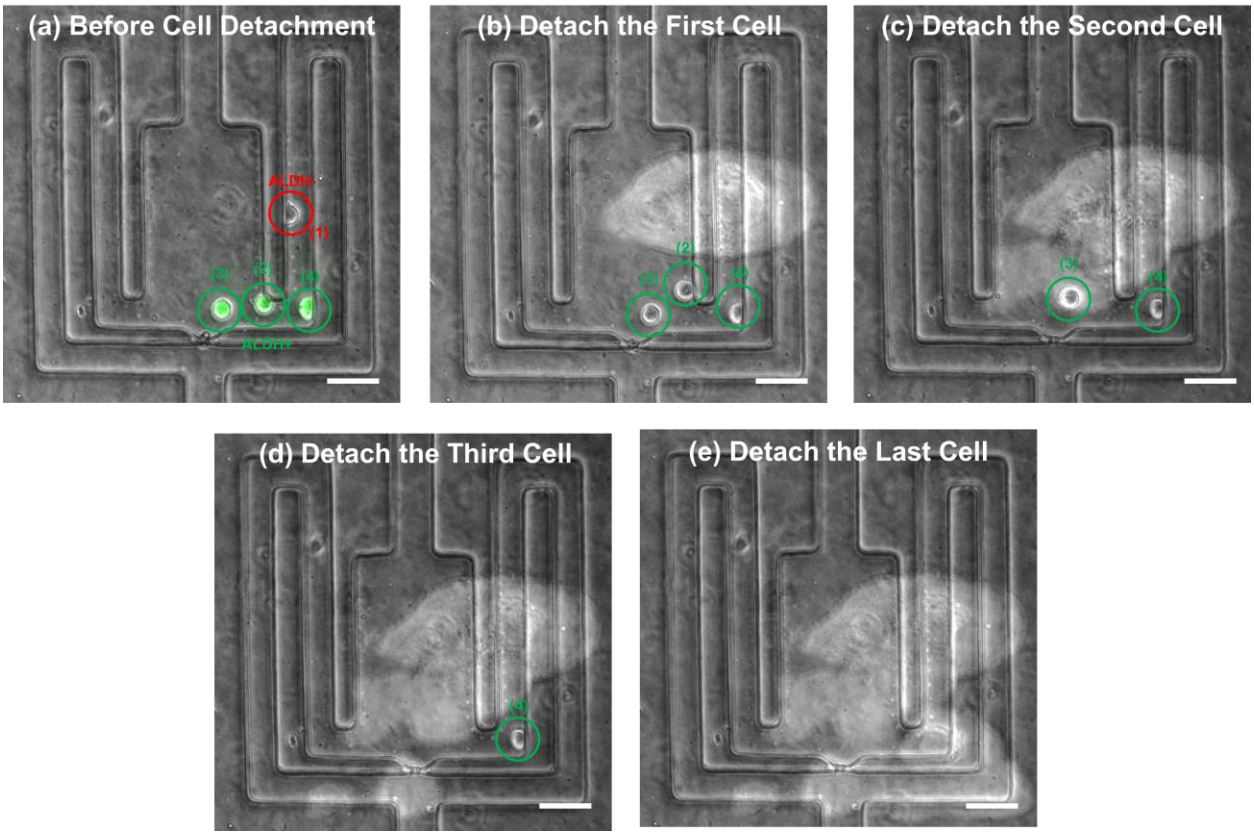
Supplementary Fig. 1: Simulations of flow velocity and pressure to evaluate cell capture scheme. 50 Pa was applied to the input (top side) of the chamber using COMSOL 5.1. (a, b) Simulations of pressure distribution before and after cell capture. (c, d) Simulations of flow velocity before and after cell capture. (e) The simulated flow pattern before cell capture. The red arrows indicating flow direction and velocity suggest that the cells are likely to be guided to the capture site and get captured. (f) After cell capture, the captured cell blocks the flow, so the next cell will be guided into the serpentine path.



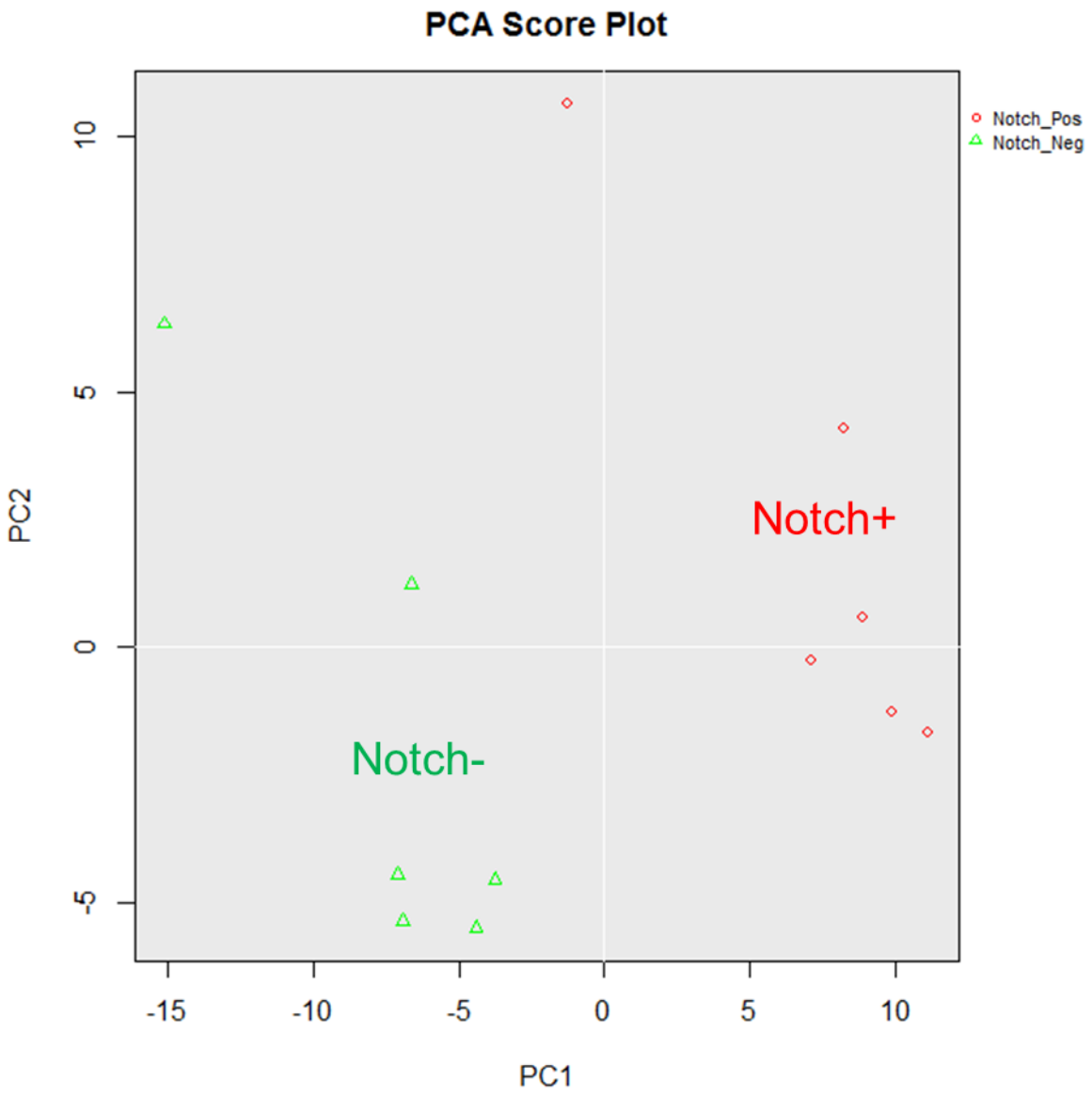
Supplementary Fig. 2. Selective single cell detachment from a CNT-PDMS composite film without a microfluidic chip on top: (a) A target cell before shear force application (within the dotted circle); (b)~(e) Each image shows the morphology change of the targeted cell resulted from the shear force produced by laser-induced micro-bubble (each bubble indicated by the arrow); (f) The detached cell after losing contact with the substrate (within the dotted circle).



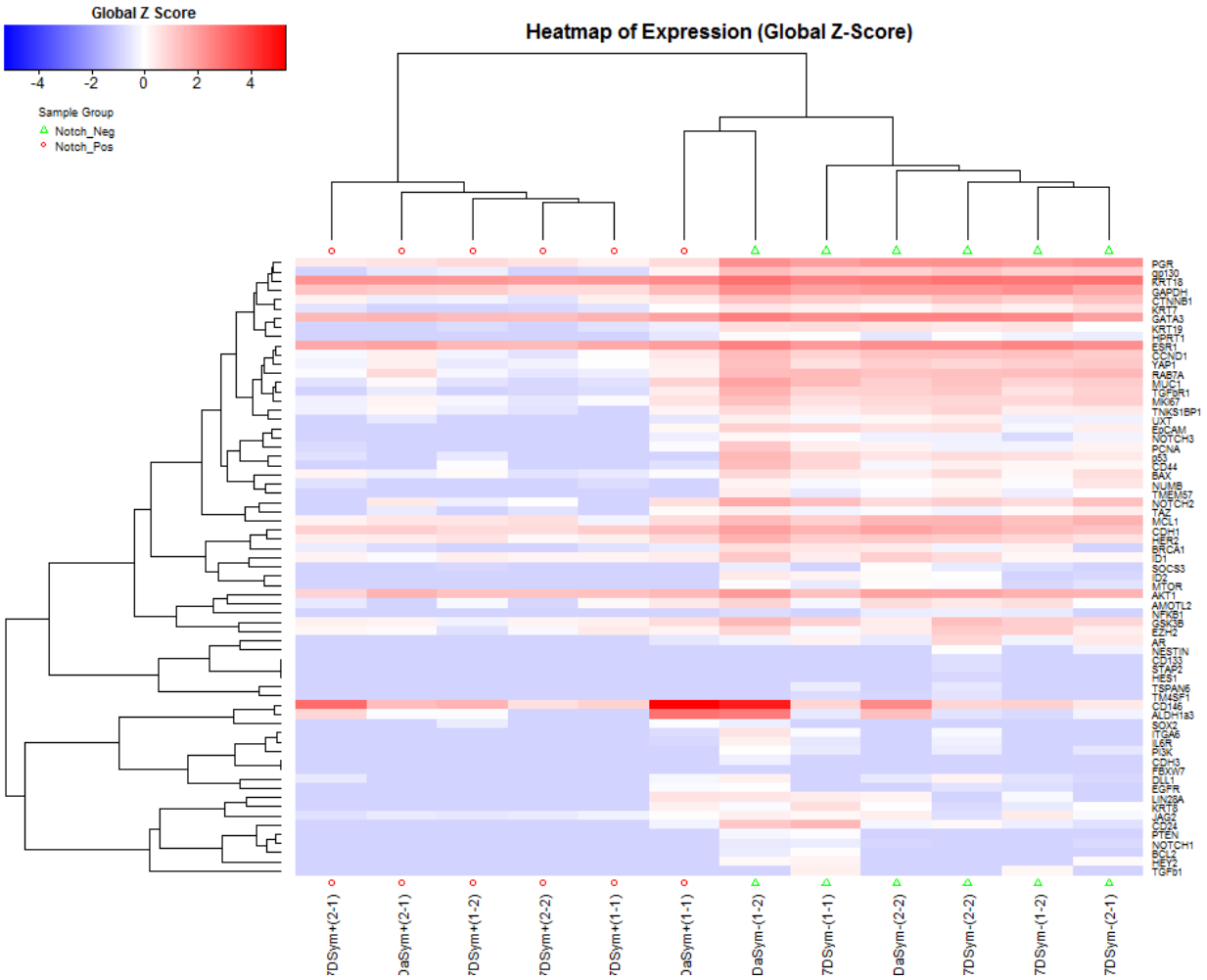
Supplementary Fig. 3. High speed (150k frames per second) camera imaging of single Skov3 cell detachment process. (scale bar: 50 μm)



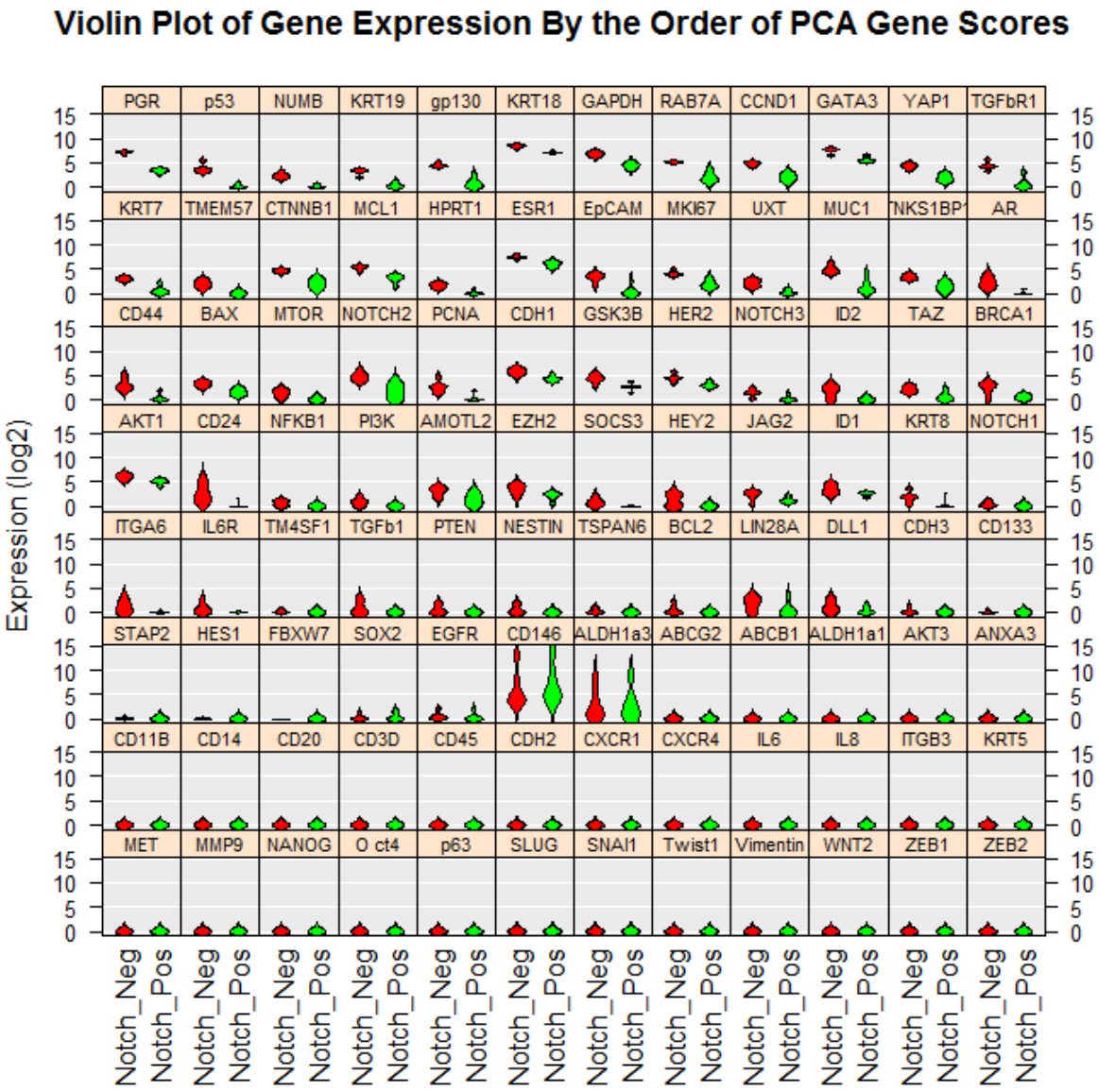
Supplementary Fig. 4. Sequential cell detachment and retrieval of 4 (3 ALDH+ and 1 ALDH-) Skov3 cells in a chamber. (scale bar: 50 μm)



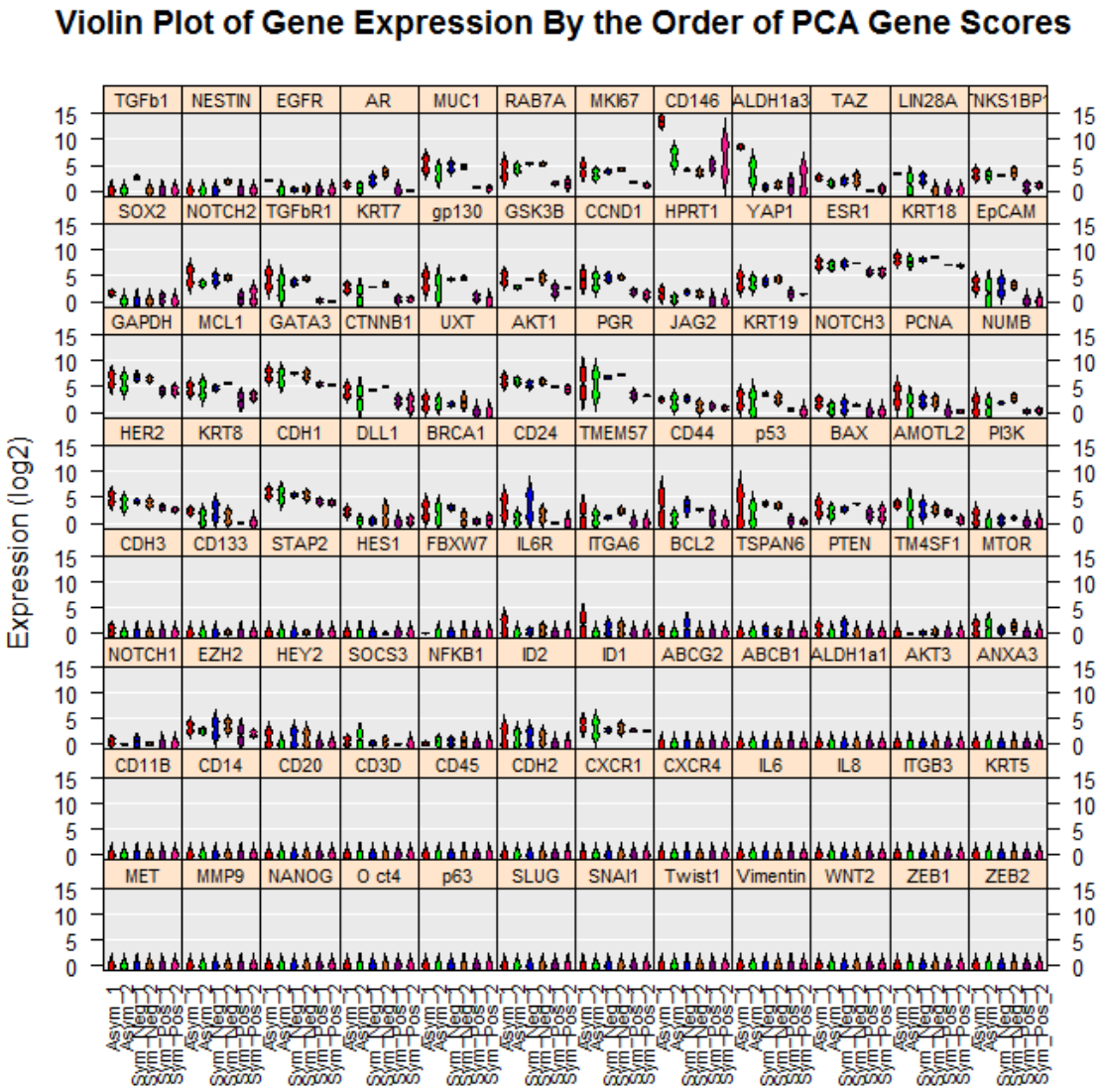
Supplementary Fig. 5. PCA of the gene expression results of Notch+ and Notch- T47D breast cancer cells using BioMark HD system and TaqMan assays. One dot represents the expression profile of a cell.



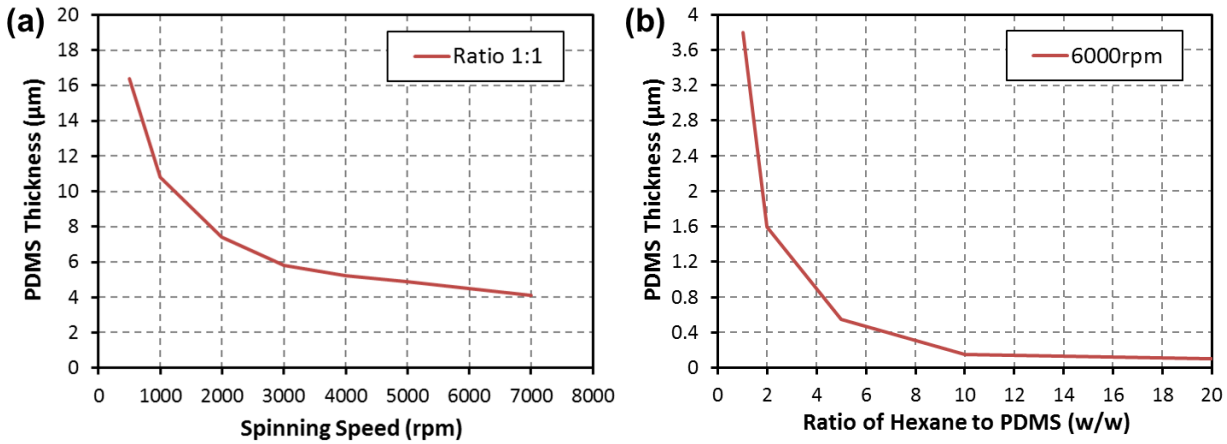
Supplementary Fig. 6. Heatmap and hierarchical cluster of the gene expression results of Notch+ and Notch- T47D breast cancer cells using BioMark HD system and TaqMan assays. In the heatmap, the red color indicates high gene expression, and the blue color indicates low gene expression. One column represents the expression profile of a cell, and one row is a gene.



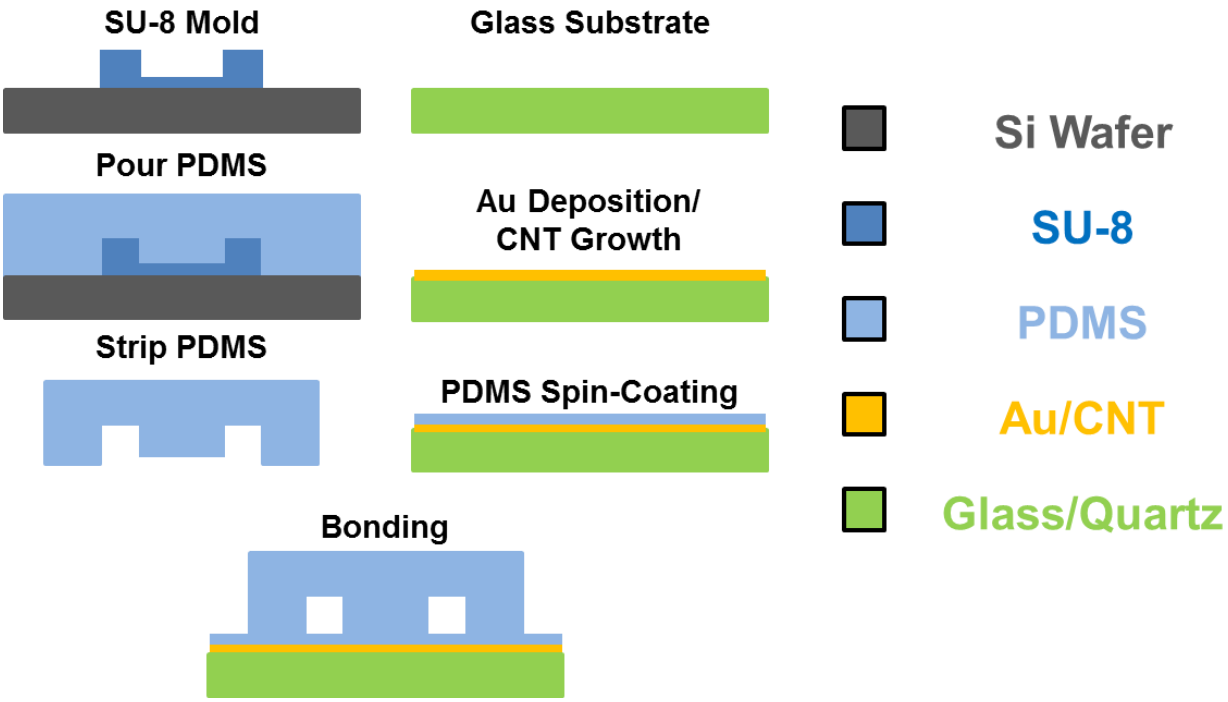
Supplementary Fig. 7. Violin plots of 96 genes of Notch+ and Notch- T47D breast cancer cells using BioMark HD system and TaqMan assays. The vertical axis indicates relative expression levels in log2 scale, and the horizontal axis indicates the distribution of cell population.



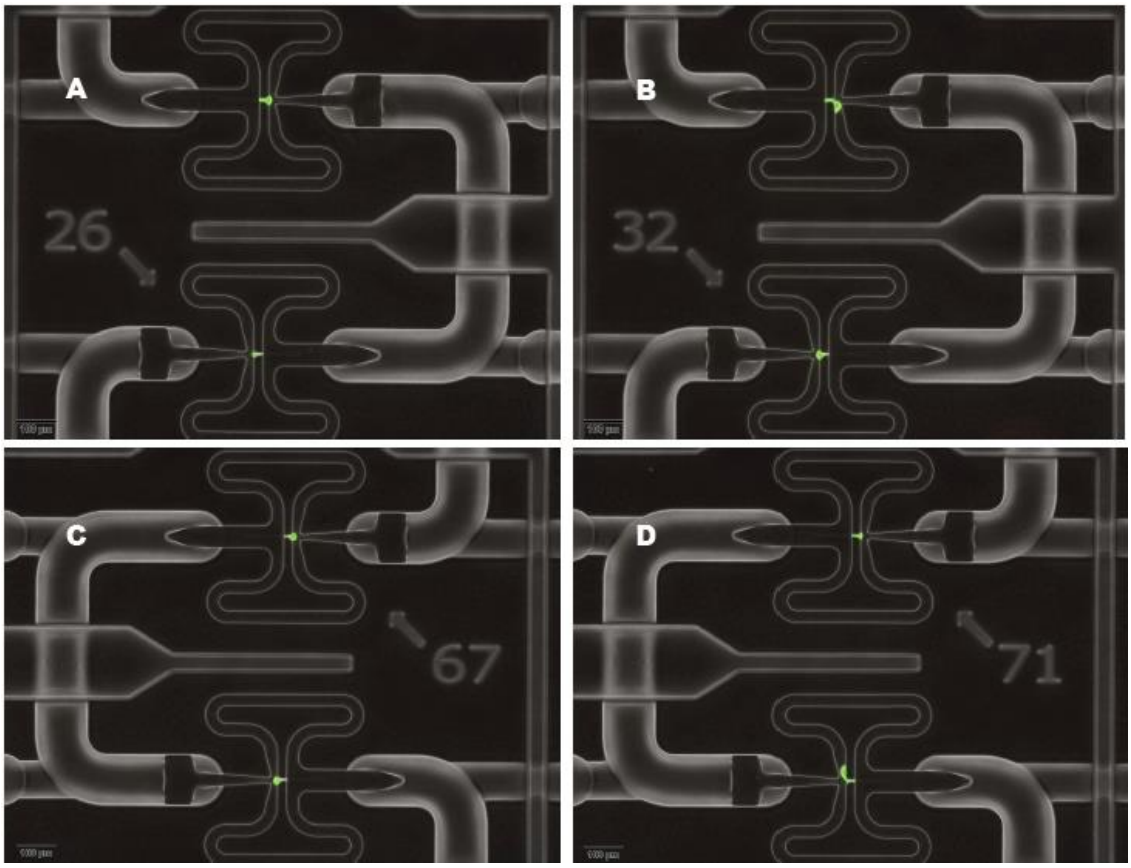
Supplementary Fig. 8. Violin plots of 96 genes of pairs of asymmetrically divided Notch+ T47D cells, symmetrically divided Notch+ and Notch- T47D cells using BioMark HD system and TaqMan assays. The vertical axis indicates relative expression levels in log2 scale, and the horizontal axis indicates the distribution of cell population.



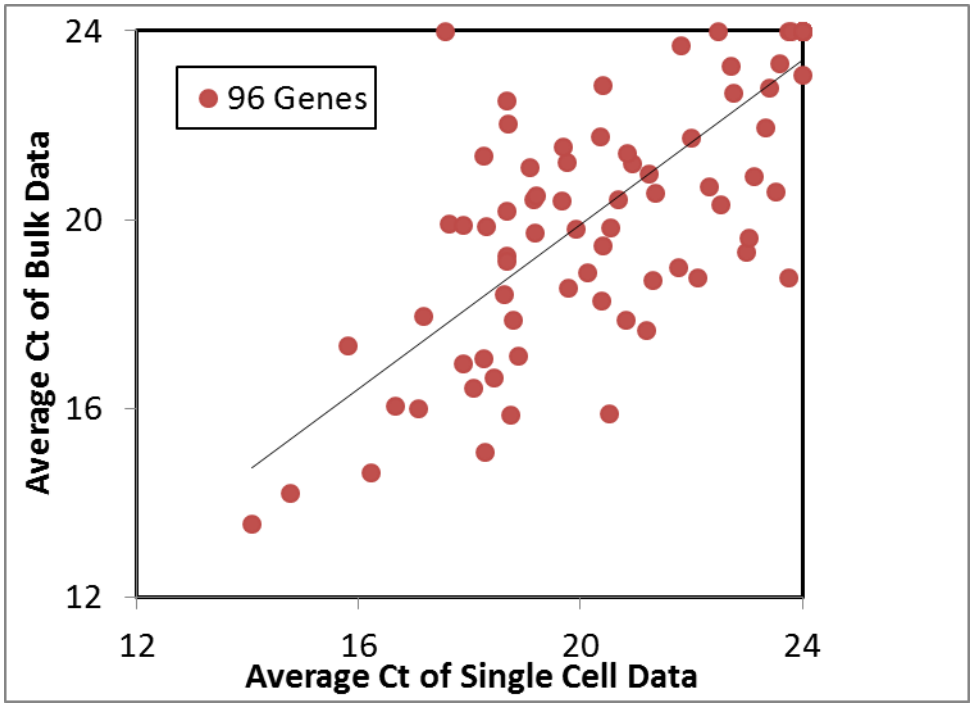
Supplementary Fig. 9: The correlation between the PDMS thickness and the spinning rate and the Hexane dilution ratio: (a) PDMS thickness versus the spinning rate, when diluted 1:1 to Hexane, and (b) PDMS thickness versus dilution ratio, when fixing to 6000rpm.



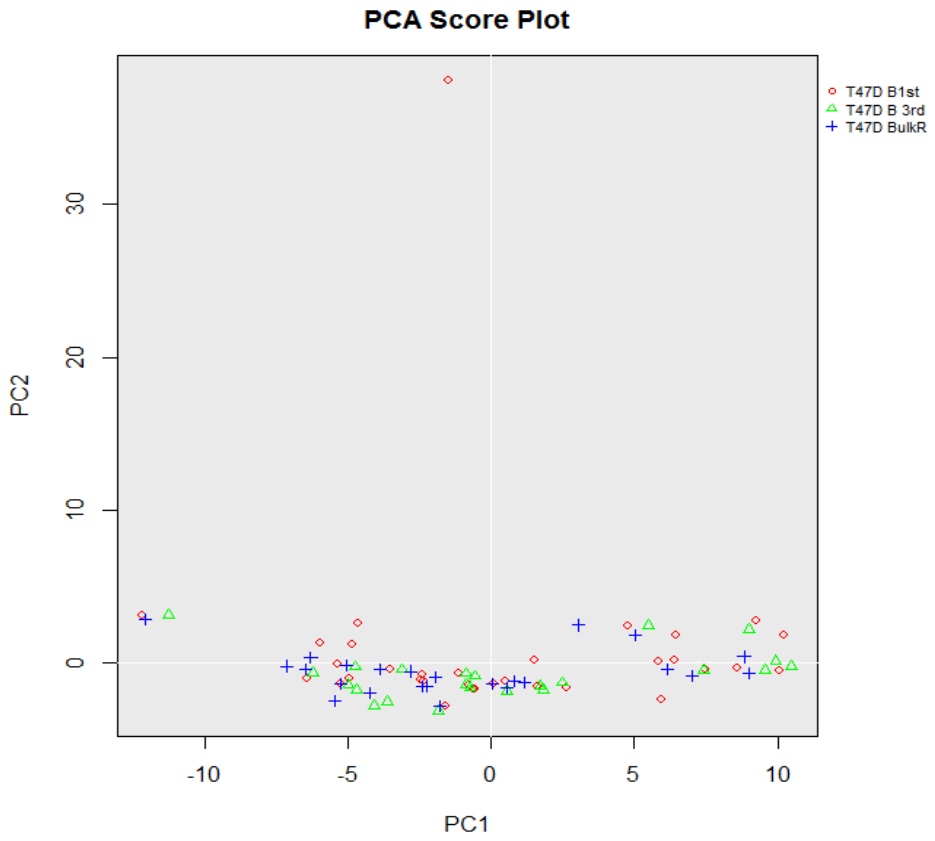
Supplementary Fig. 10: Fabrication process of the selective single cell retrieval platform.



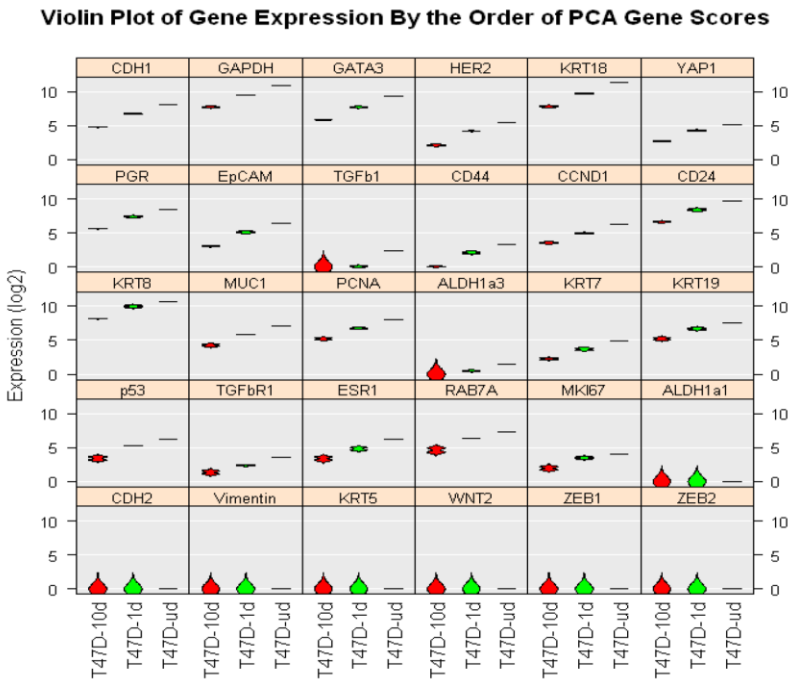
Supplementary Fig. 11. Representative images of single breast cancer cells isolated within the C1 chip. (a) Large versus small size round shape single cells, (b) elongated versus round shape single cells, (c) similar size round shape single cells (d) and small round shape versus large elongated single cells.



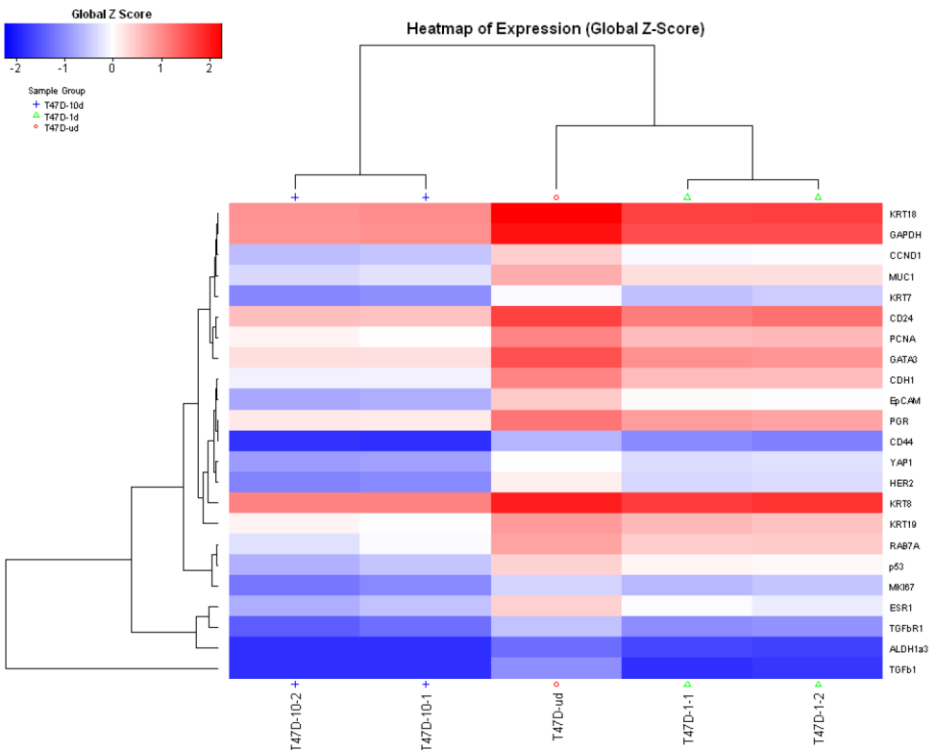
Supplementary Fig. 12. Correlation between single T47D cell (n = 28) expression data and bulk T47D (5,000 cells each sample, n = 3) expression data (both normalized to the geometric mean of GAPDH and RAB7A). The average Ct of 96 genes are highly correlated (correlation coefficient r = 0.79) between single cell and bulk data.



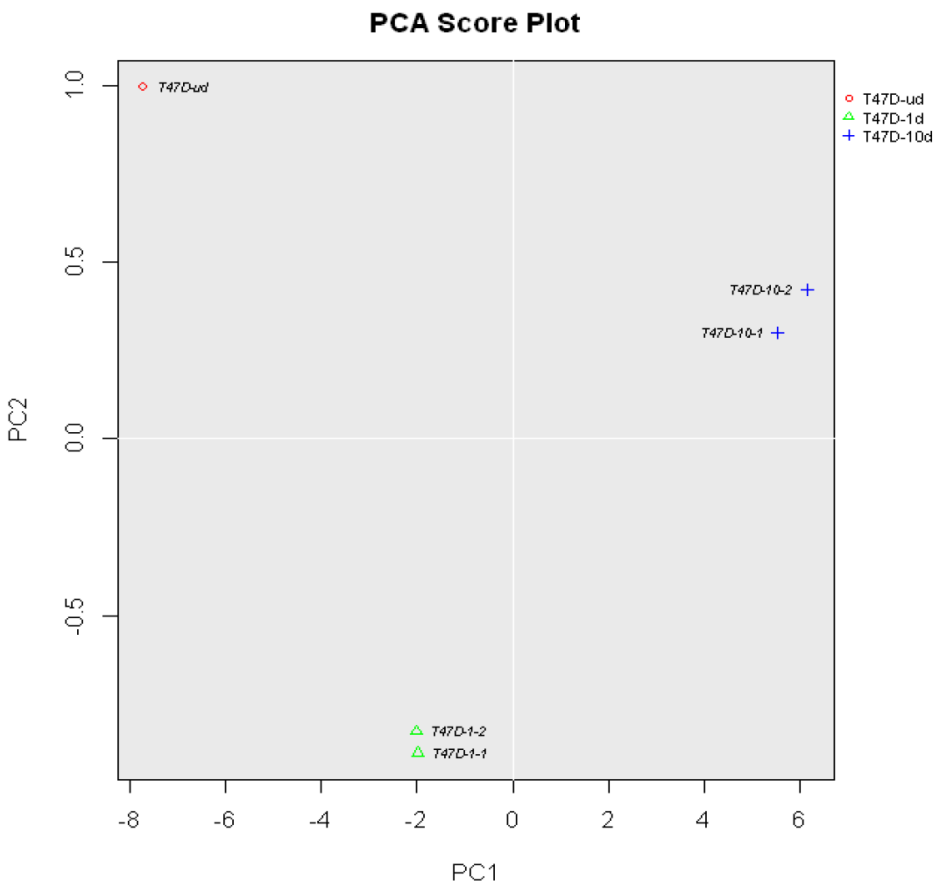
Supplementary Fig. 13. Principal component analysis (PCA) plot showing log2 gene expression data of single cells from T47D breast cancer cell line. T47D B1st (Red) and T47D BulkR (Blue) are the same single cells isolated by C1 and analyzed by BioMark HD in 2 separate RT-qPCR experiments as technical replicates. T47D B3rd (Green) and T47D B1st (Red) are biological replicates of 2 independent C1/BioMark HD experiments. The vertical axis indicates relative expression levels in log2 scale, and the horizontal axis indicates the distribution of cell population.



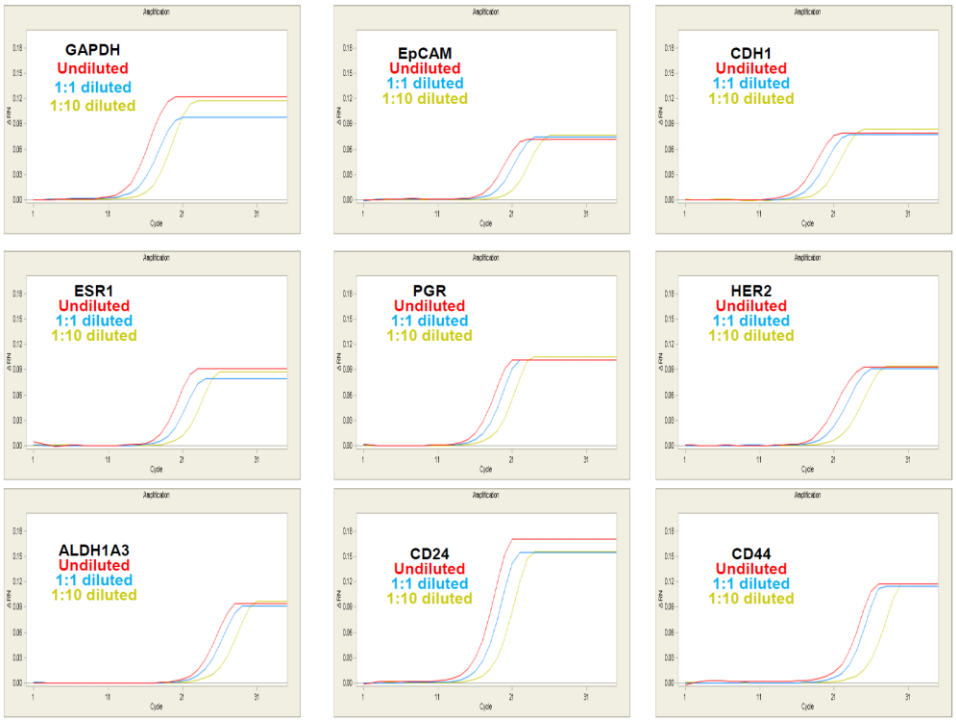
Supplementary Fig. 14. Violin plot of the gene expression results of undiluted (ud), 1:1 diluted (1d), and 1:10 diluted (10d) total RNA extracted from T47D breast cancer cell line using BioMark HD system and TaqMan assays. The vertical axis indicates relative expression levels in log2 scale, and the horizontal axis indicates the distribution of cell population.



Supplementary Fig. 15. Heatmap of the gene expression results of undiluted (ud), 1:1 diluted (1d), and 1:10 diluted (10d) total RNA extracted from T47D breast cancer cell line using BioMark HD system and TaqMan assays. In the heatmap, the red color indicates high gene expression, and the blue color indicates low gene expression. One column represents the expression profile of a sample, and one row is a gene.



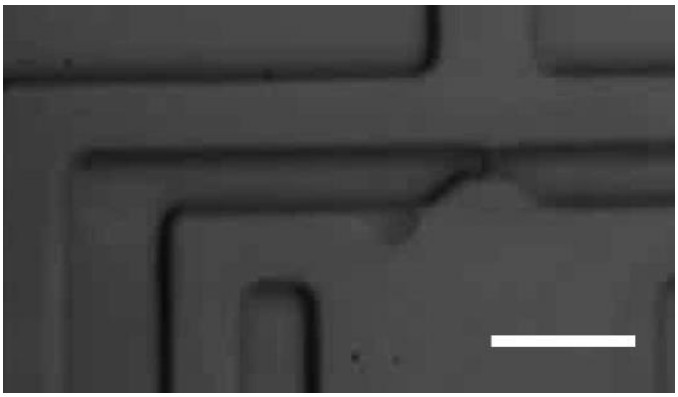
Supplementary Fig. 16. PCA of the gene expression results of undiluted (ud), 1:1 diluted (1d), and 1:10 diluted (10d) total RNA extracted from T47D breast cancer cell line using BioMark HD system and TaqMan assays. One dot represents the expression profile of a gene.



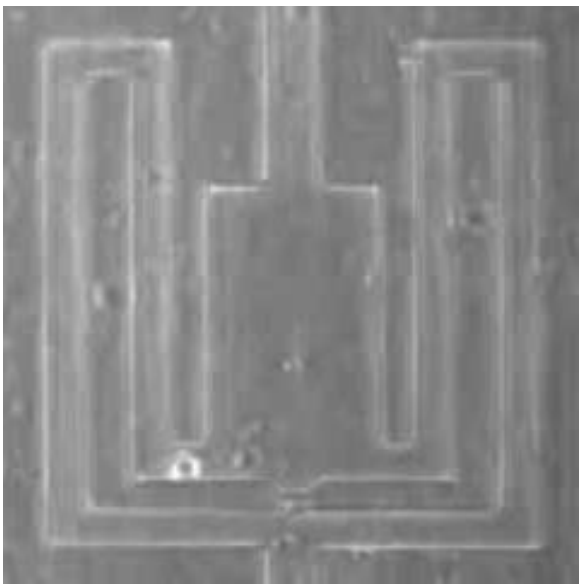
Supplementary Fig. 17. Amplification plots of the critical genes of undiluted (ud), 1:1 diluted (1d), and 1:10 diluted (10d) total RNA extracted from T47D breast cancer cell line using BioMark HD system and TaqMan assays.

Supplementary Video 1. A MDA-MB-231 cell was loaded into the chamber by gravity flow, and the first coming cell was captured at the capture site.

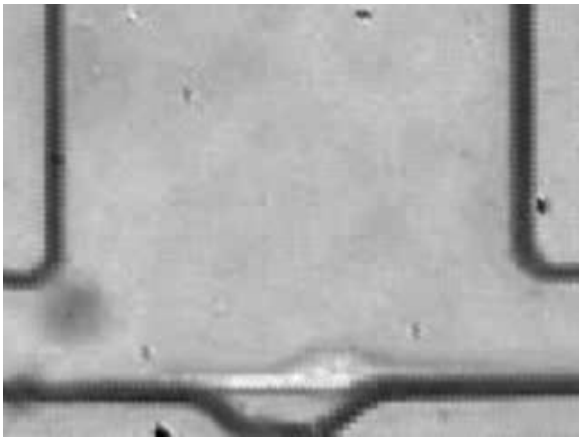
Supplementary Video 2. A MDA-MB-231 cell was loaded into the chamber by gravity flow. As the capture site was occupied by a captured cell, and the next coming cell flow to downstream through serpentine path.



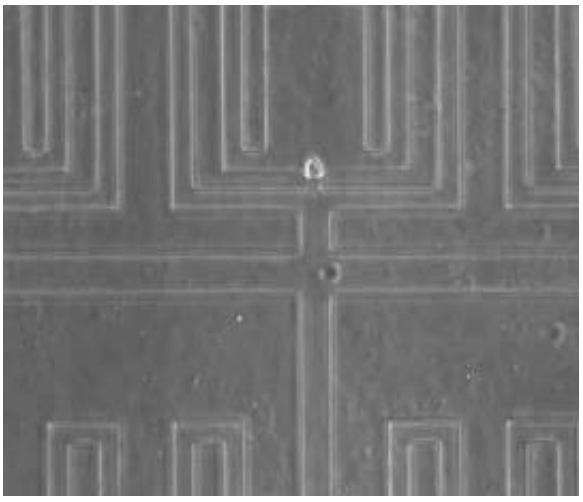
Supplementary Video 3. High speed (150k frames per second) camera imaging of single Skov3 cell detachment.



Supplementary Video 4. An example of single T47D cell detachment.



Supplementary Video 5. An example of partial MDA-MB-231 cell detachment.



Supplementary Video 6. An example of single T47D cell retrieval.