Electronic Supplementary Material (ESI) for Lab on a Chip.

Title:

Single Cell Dual Adherent-Suspension Co-Culture Micro-Environment for Studying Tumor-Stromal Interactions

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Supplementary Tables

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

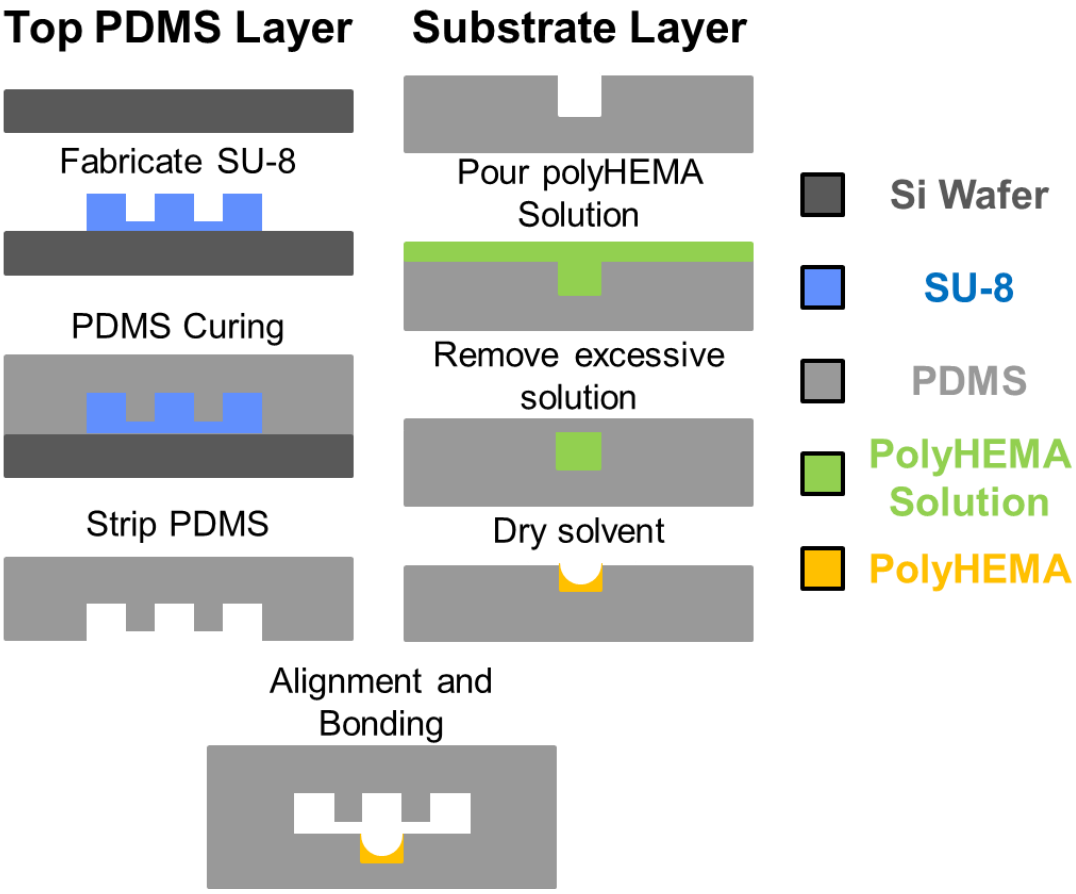
Supplementary Table 2. The significantly different genes between mono-cultured T47D and co-cultured T47D cells.

GeneID	P-Value	T47D_Mono.Average	T47D_Co.Average
KRT8	1.12E-11	6.499923	3.933005
GATA3	1.35E-11	10.1518	8.411626
STAP2	3.79E-11	1.686678	0.22912
ANXA3	1.26E-10	0	4.82124
PI3K	5.45E-10	2.287886	0.786667
RAB7A	6.68E-10	6.720044	5.250553
NUMB	1.66E-09	3.416033	1.718079
TNKS1BP1	1.60E-08	3.94843	2.214316
KRT18	2.85E-08	8.774339	7.566195
HES1	6.28E-08	3.101816	1.386066
HER2	8.57E-07	3.783676	2.242492
MUC1	9.26E-07	6.016341	4.896981
NOTCH3	1.60E-06	1.86625	0.754887
CDH1	1.51E-05	7.267235	6.347858
UXT	1.78E-05	3.2059	2.154927
MTOR	3.19E-05	2.229556	1.159141
CTNNB1	4.85E-05	5.813502	4.985064
MKI67	8.85E-05	0.203386	1.79967
KRT19	0.000147	5.695987	4.566369
SOCS3	0.00022	3.07186	1.795577
CD24	0.00033	4.988667	3.551879
MCL1	0.000817	5.789805	5.027195
BAX	0.000927	3.141727	1.894075
PTEN	0.00323	2.354125	1.641271
DLL1	0.004232	0.088481	0.872926
PGR	0.00432	1.186971	2.392999
JAG2	0.005557	0.594469	1.414207
CXCR4	0.005948	1.949017	1.187916
ITGA6	0.011887	1.534064	0.777038
AMOTL2	0.015817	0.228205	0.776759
TSPAN6	0.016113	0.366471	0.07542
O ct4	0.023234	3.24693	3.681141
HPRT1	0.024165	0.440148	0.943632
CDH3	0.035109	0.213805	0.040821
NOTCH2	0.041917	3.866909	3.33749

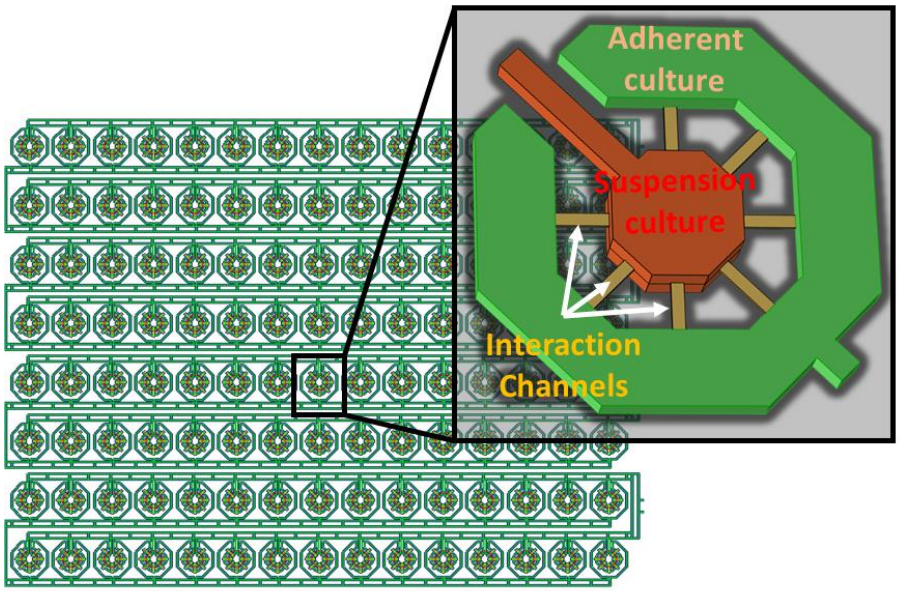
Supplementary Table 3. The significantly different genes between MKI67 high and MKI67 low cells in co-culture group.

GeneID	P-Value	MKI67+.Average	MKI67-.Average
MKI67	5.90E-14	3.904091	0
EZH2	3.08E-07	3.110625	0.281208
JAG2	6.42E-05	2.450704	0.649195
CCND1	0.000245	4.777958	6.013382
PCNA	0.000464	6.096981	3.268994
BRCA1	0.00078	3.258604	0.913685
ANXA3	0.0019	2.885846	6.501779
NOTCH1	0.003295	2.031445	0.843698
BAX	0.008143	2.658863	1.215017
HPRT1	0.029467	1.574787	0.702613
ID1	0.049499	4.654944	3.797625

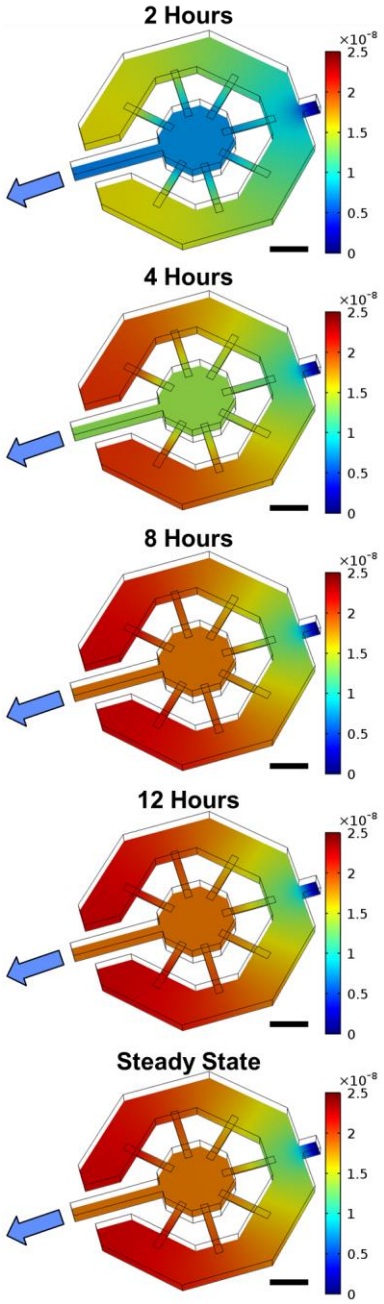
Supplementary Figures



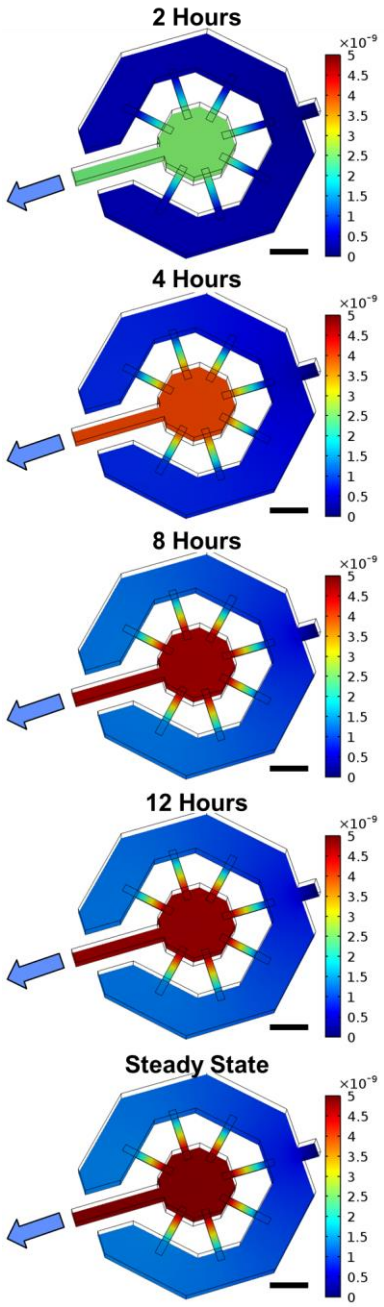
Supplemental Fig. 1. Fabrication process of the dual adherent-suspension co-culture micro-environment.



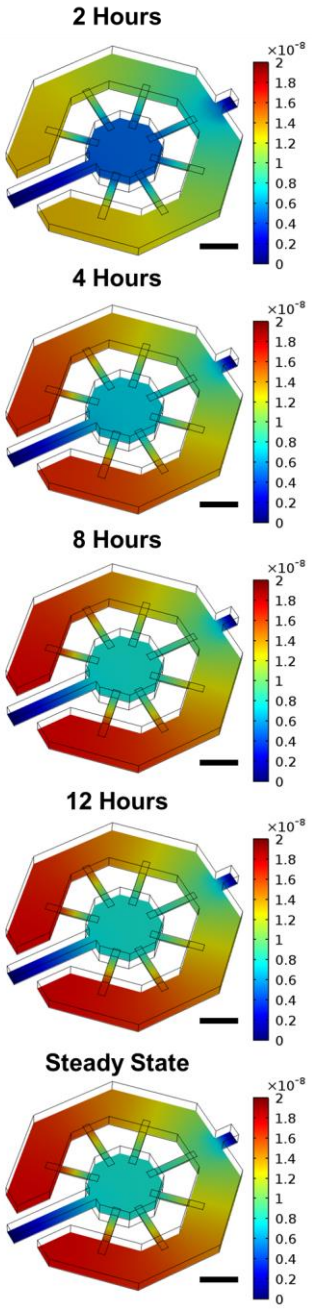
Supplementary Fig. 2: The presented dual culture platform. Cancer cells are loaded into a suspension chamber for clonal sphere formation while the stromal cells are cultured in the adherent chamber in close proximity for interaction through small connecting channels.



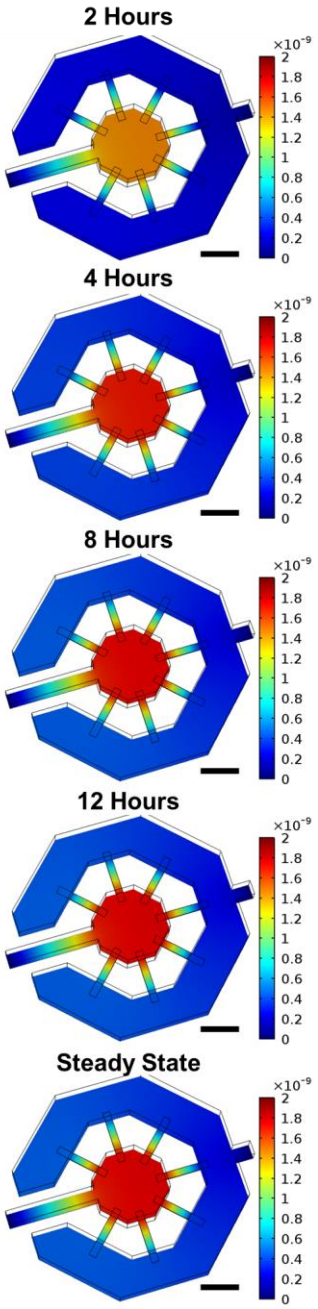
Supplemental Fig. 3. Simulations of the distribution of the concentration (mole/L) of the proteins secreted by fibroblast when 50 Pa was applied to the adherent port of the chamber (the flow condition right after media exchange) using COMSOL 5.1. We assume that one cell secretes 1000 proteins per second, 10 fibroblasts are captured in the outer chamber, and the protein diffusion coefficient is 1^{-10} m^2/s.[1-4] (Scale bar: 100 μm)



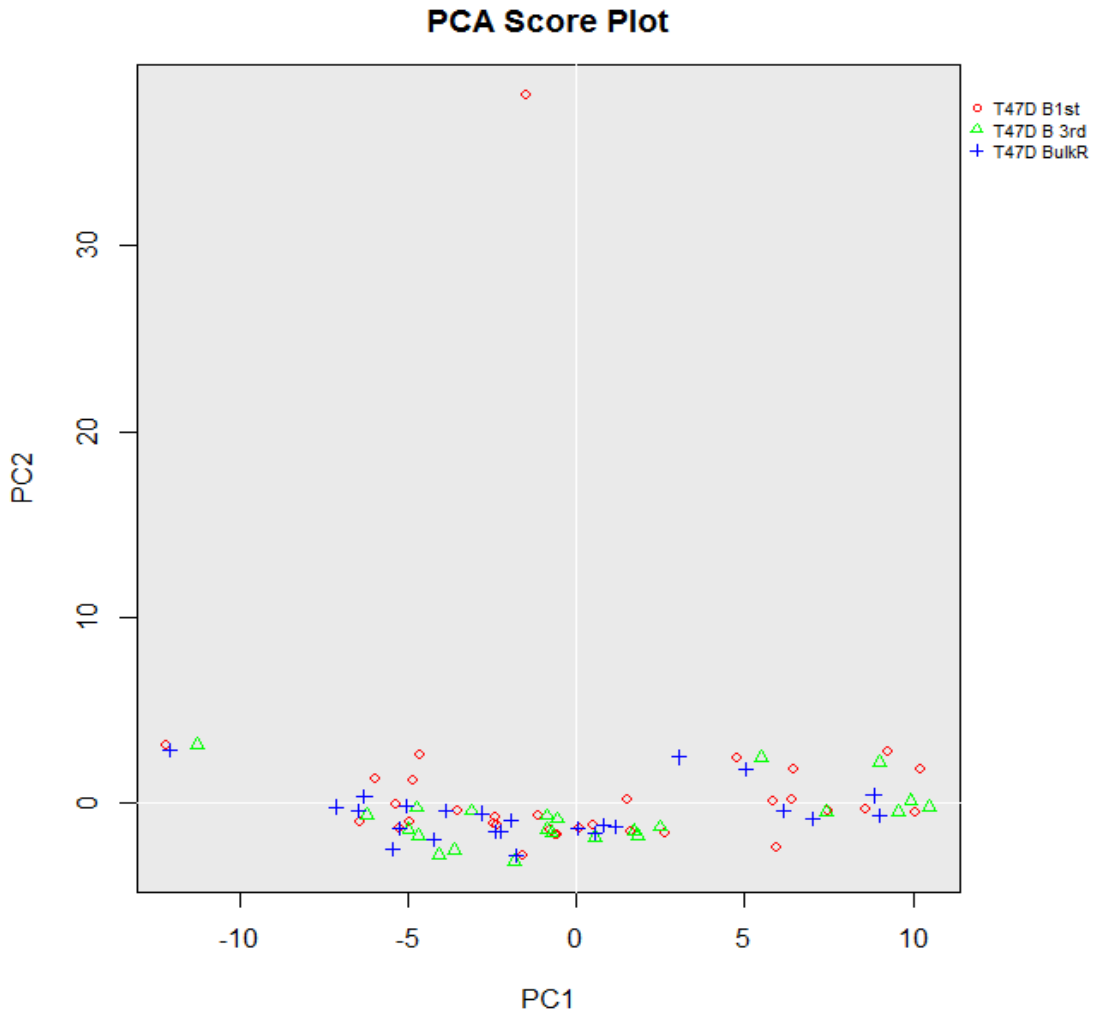
Supplemental Fig. 4. Simulations of the distribution of the concentration (mole/L) of the proteins secreted by cancer cell when 50 Pa was applied to the adherent port of the chamber (the flow condition right after media exchange) using COMSOL 5.1. We assume that one cell secretes 1000 proteins per second, 1 cancer cell is captured in the inner chamber, and the protein diffusion coefficient is 1^{-10} m^2/s. [1-4] (Scale bar: 100 μm)



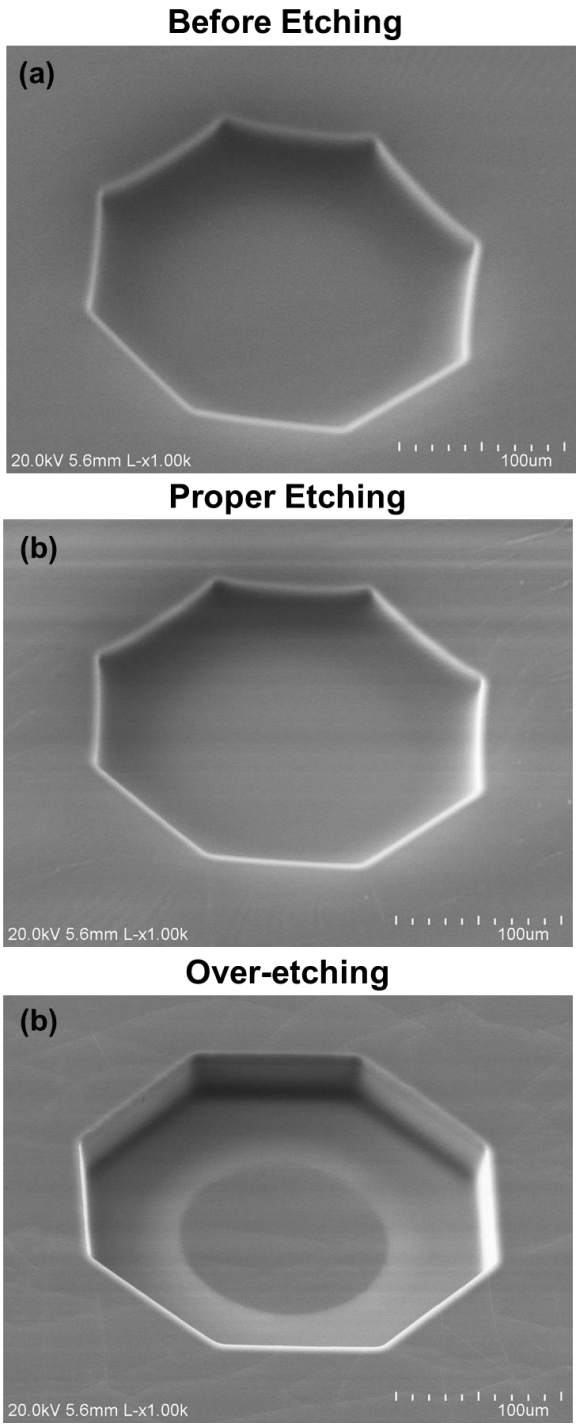
Supplemental Fig. 5. Simulations of the distribution of the concentration (mole/L) of the proteins secreted by fibroblast when there is no flow using COMSOL 5.1. We assume that one cell secretes 1000 proteins per second, 10 fibroblasts are captured in the outer chamber, and the protein diffusion coefficient is 1^{-10} $m^2/s.$ $^{1-4}$ (Scale bar: 100 μm)



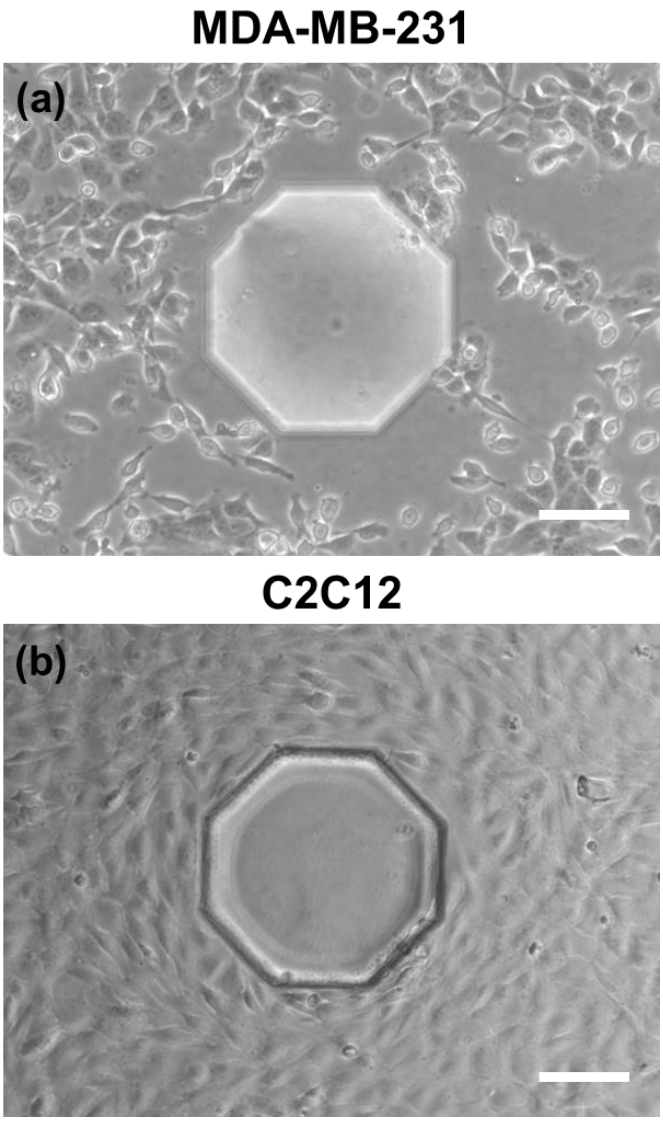
Supplemental Fig. 6. Simulations of the distribution of the concentration (mole/L) of the proteins secreted by cancer cell when there is no flow using COMSOL 5.1. We assume that one cell secretes 1000 proteins per second, 1 cancer cell is captured in the inner chamber, and the protein diffusion coefficient is 1^{-10} m^2/s. [1-4] (Scale bar: 100 μm)



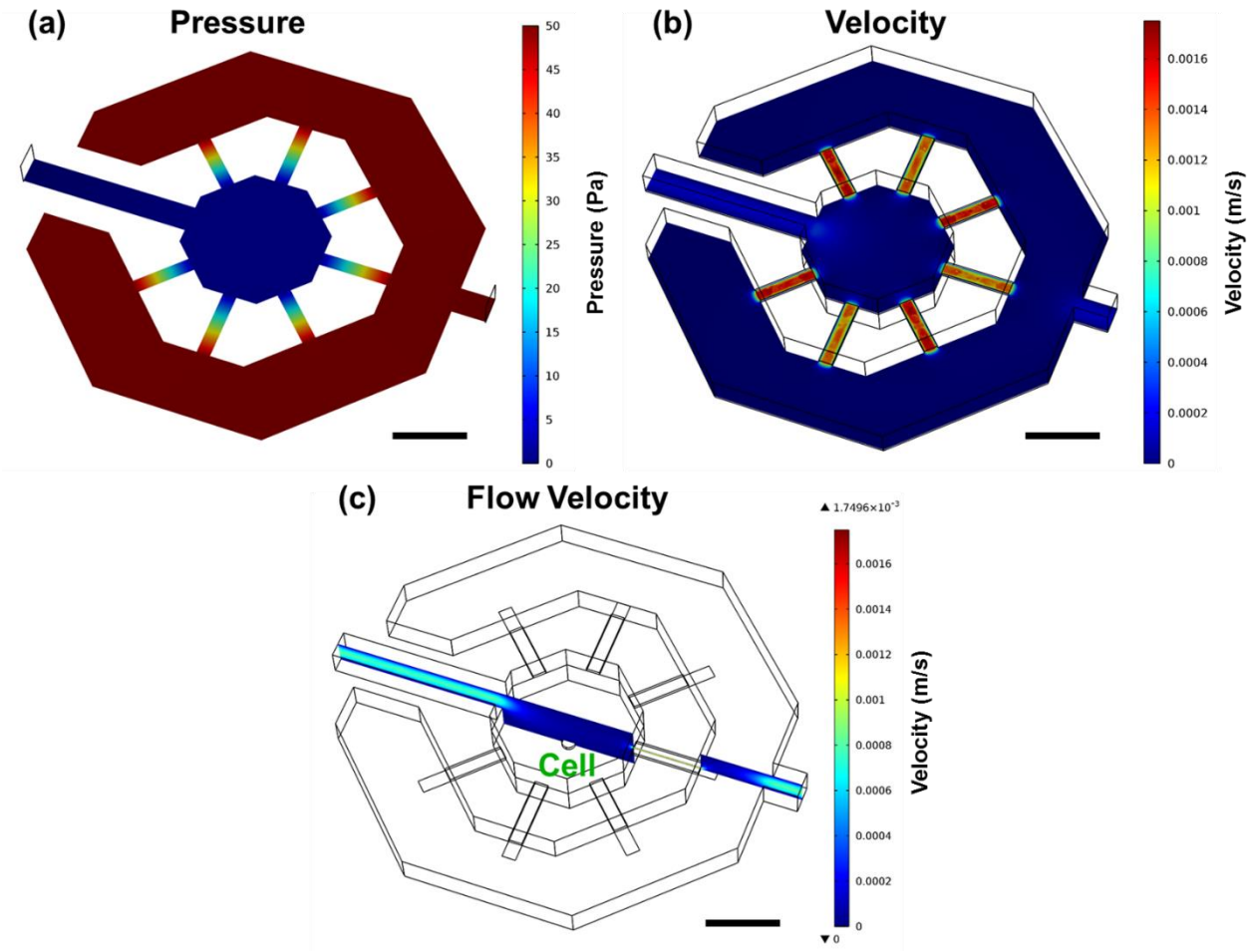
Supplemental Fig. 7. Principal component analysis (PCA) plot showing log2 gene expression data of single cells from T47D breast cancer cell line. T47D B1st (Red) and T47D BulkR (Blue) are the same single cells isolated by C1 and analyzed by BioMark HD in 2 separate RT-qPCR experiments as technical replicates. T47D B3rd (Green) and T47D B1st (Red) are biological replicates of 2 independent C1/BioMark HD experiments.



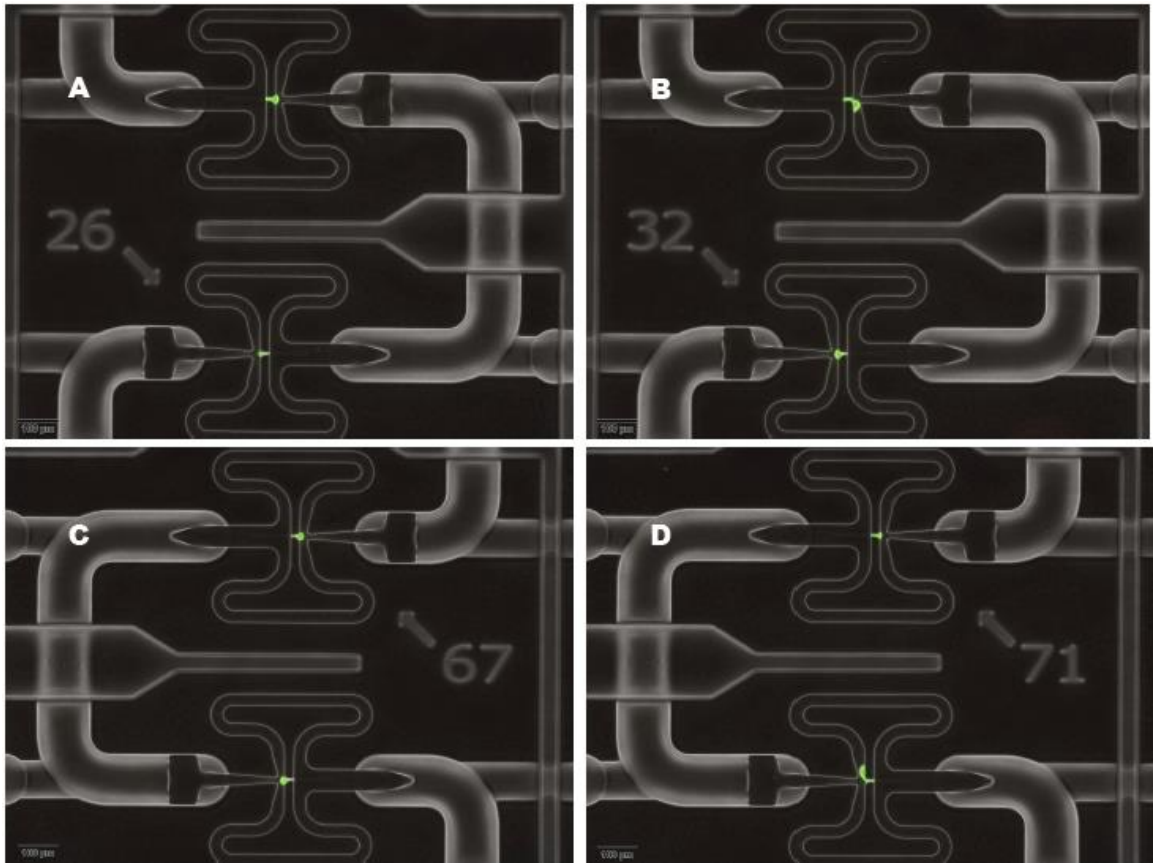
Supplemental Fig. 8. The plasma etching process to remove residual polyHEMA. (a) Before Etching, (b) Proper etching only removes the un-desired polyHEMA residues. (b) Over-etching can etch the polyHEMA in the indented well and expose the PDMS substrate.



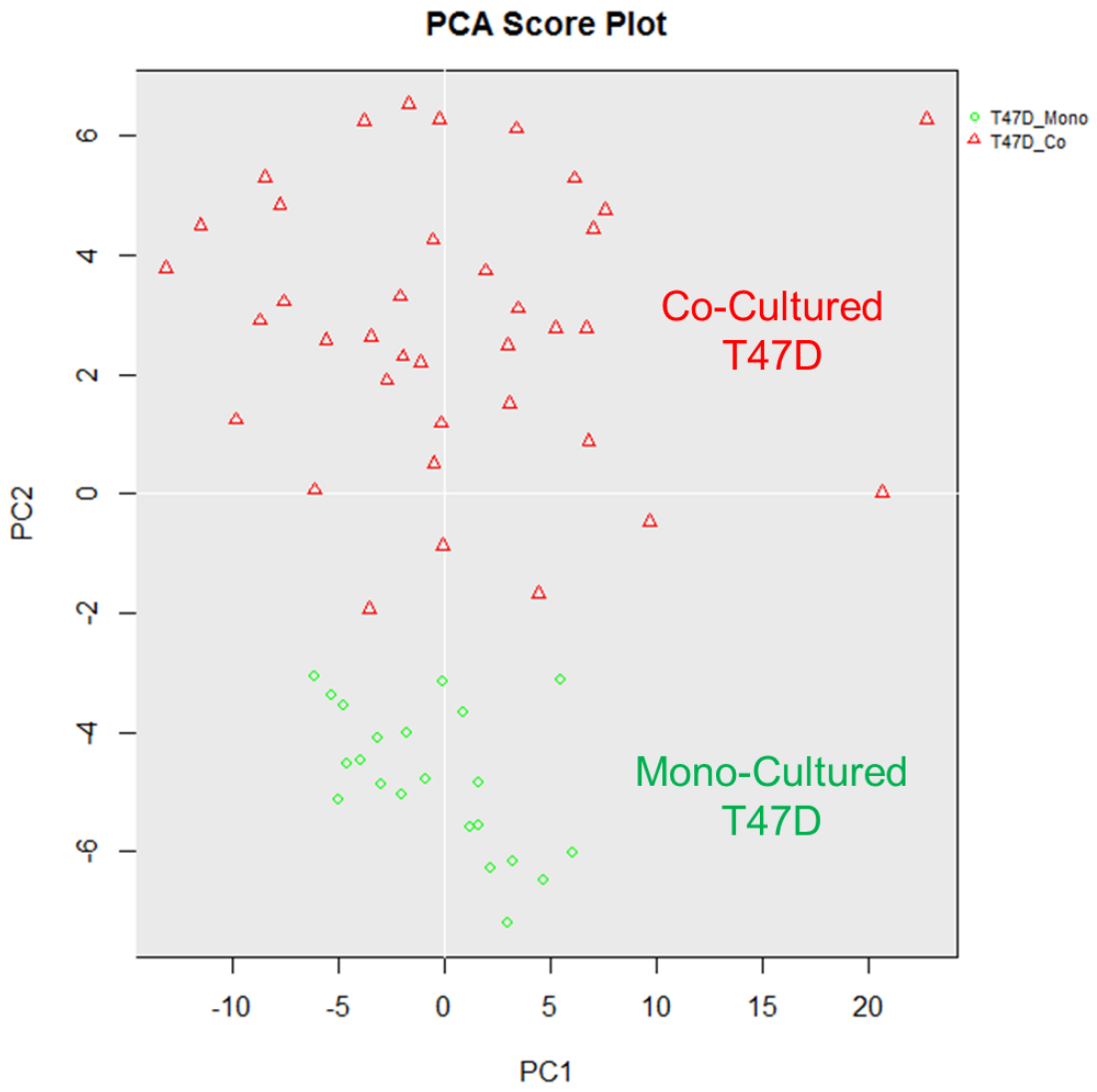
Supplemental Fig. 9. The selectivity of the adherent-suspension co-culture micro-environment of (a) MDA-MB-231 and (b) C2C12 cells. For both cell lines, no cell adhere in the polyHEMA-coated indented well, but cells can adhere on the un-coated. (scale bar: 100 μm)



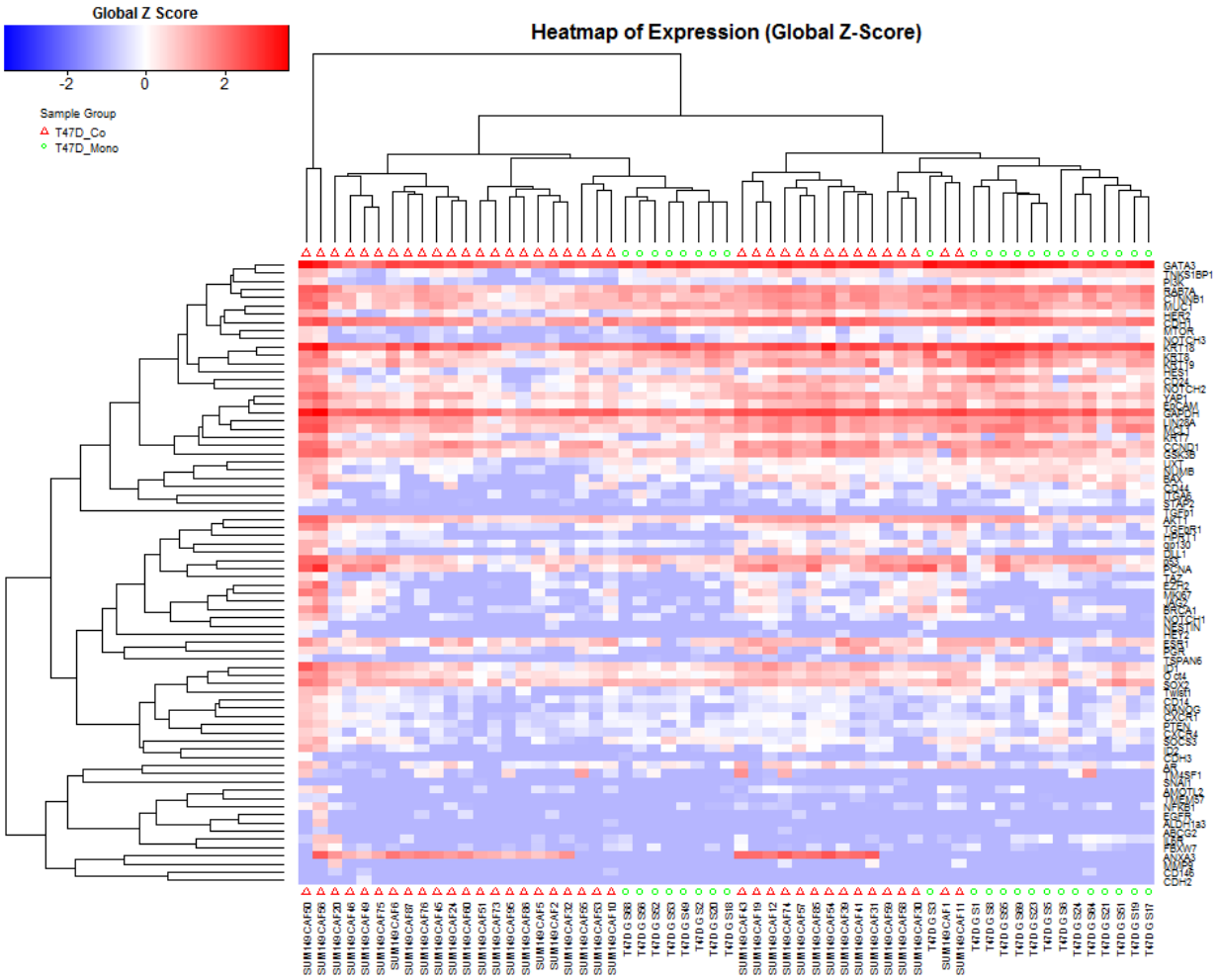
Supplemental Fig. 10. COMSOL simulations of the cell loading process: (a) pressure, (b) flow velocity in the XY-plane of 1.5 µm height (the half of the height of the interaction channel), and (c) the flow velocity in the XZ-plane. 50 Pa was applied to the adherent port of the chamber to simulate the flow condition right after media exchange. The results suggest that once a cell settles into the indented well, it is unlikely to be washed out by reversing flow. (Scale bar: 100 µm)



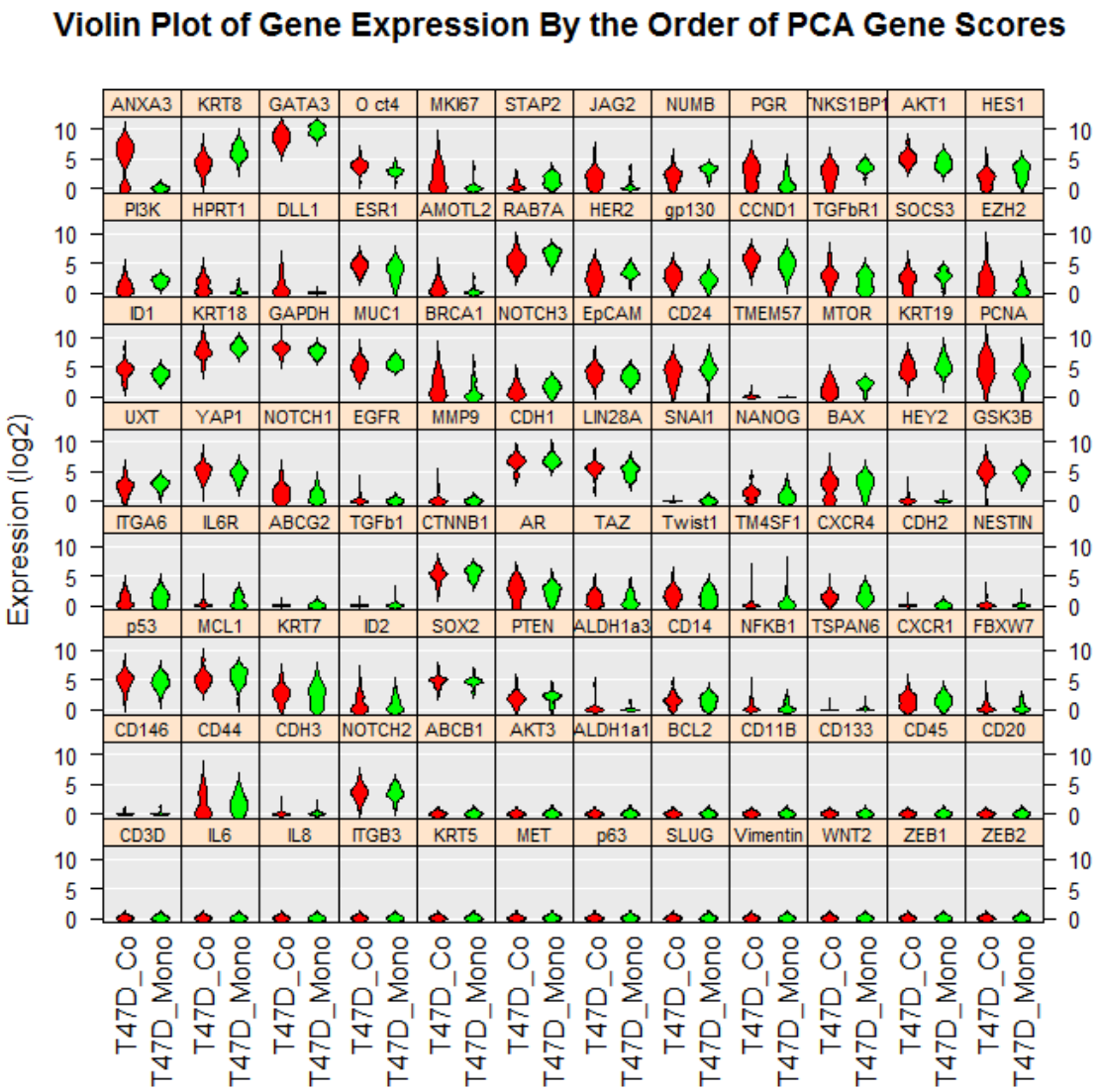
Supplemental Fig. 11. Representative images of single breast cancer cells isolated within the C1 chip. Cells of different sizes and morphology can be captured on-chip.



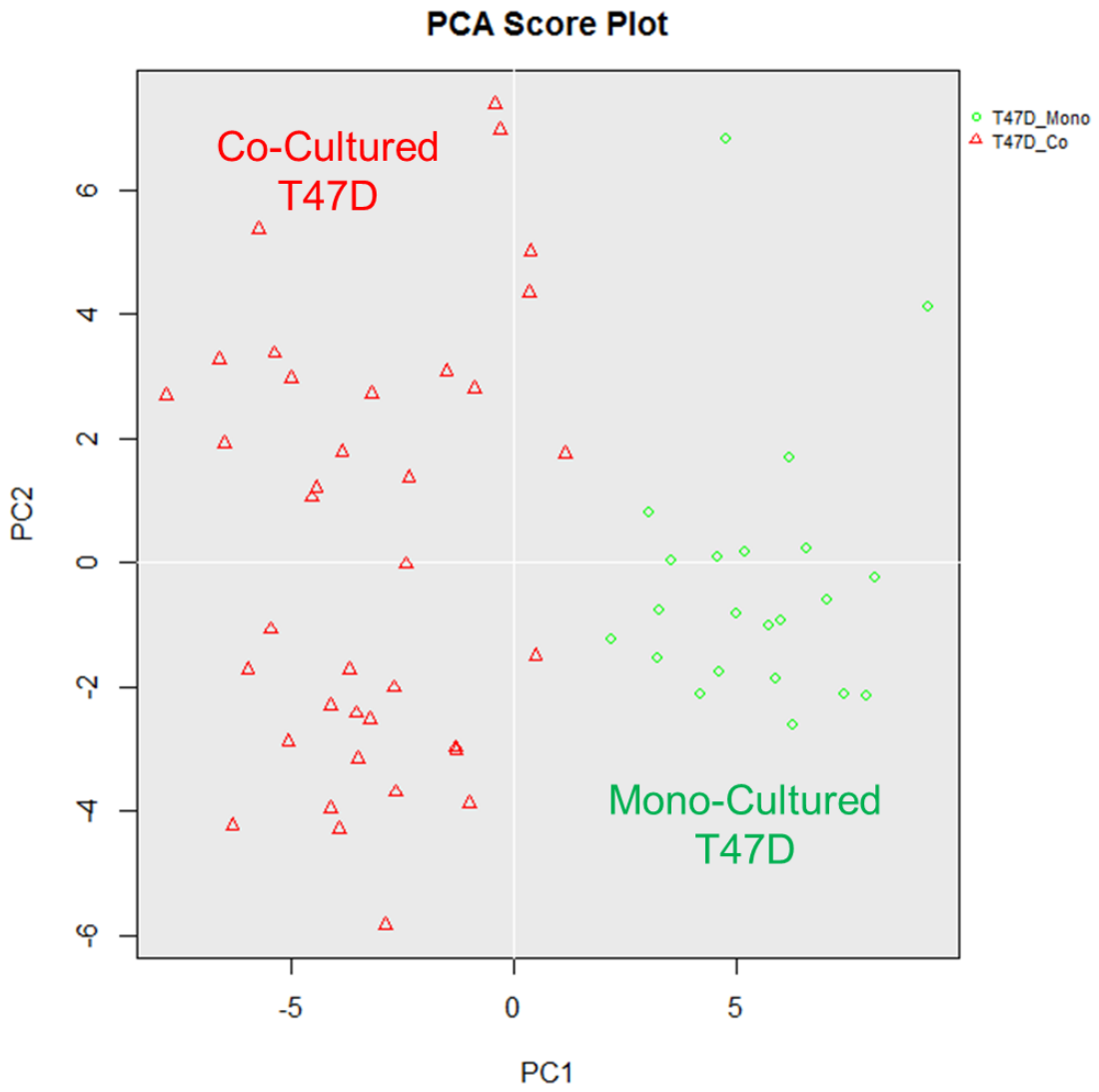
Supplementary Fig. 12. PCA clustering of single cell expression analysis for co-cultured (red, N=37) and mono-cultured (green, N=22) T47D cells compared. The results were not normalized.



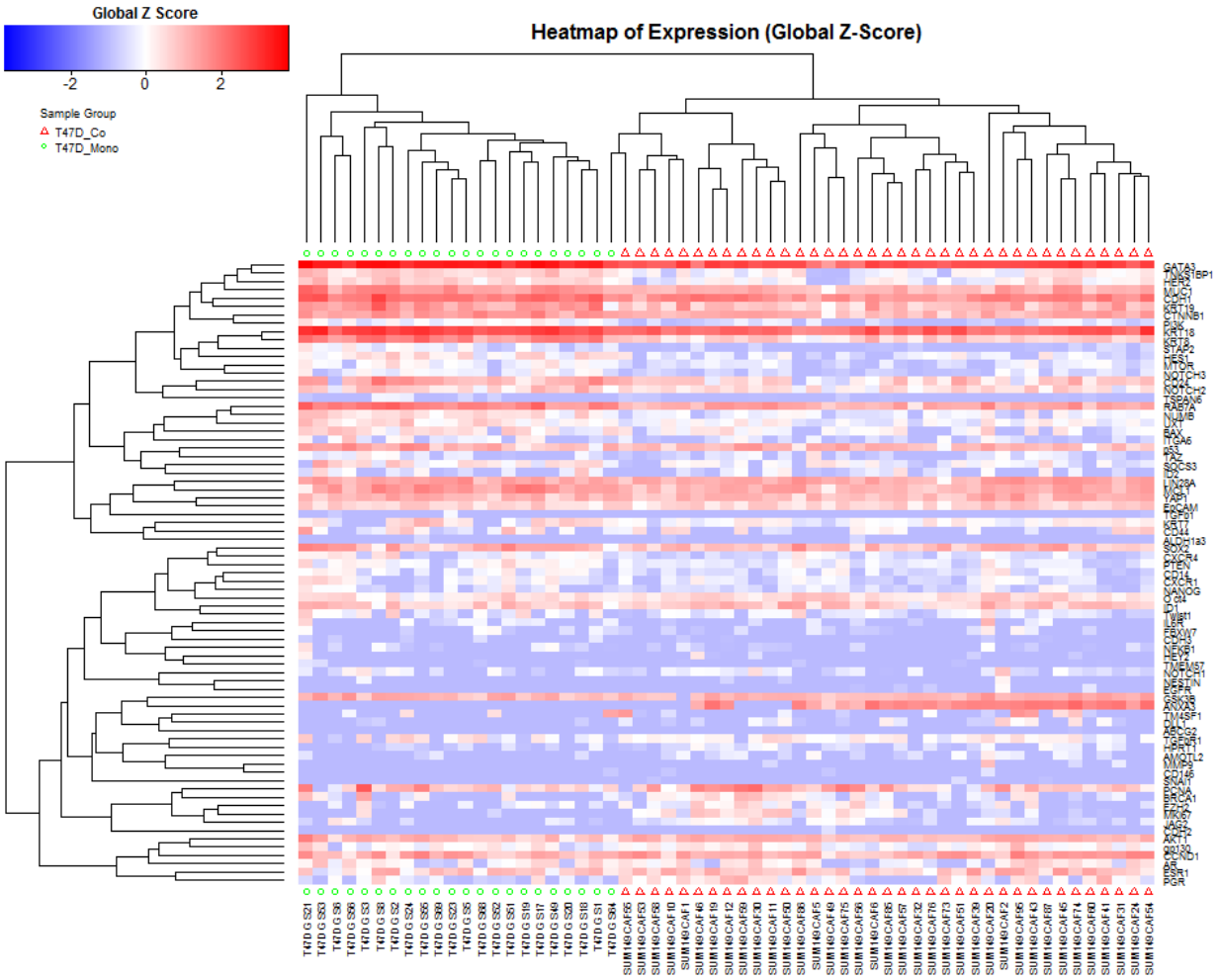
Supplementary Fig. 13. Heatmap hierarchical clustering of single cell expression analysis for between co-cultured (red, N=37) and mono-cultured (green, N=22) T47D cells. The results were not normalized.



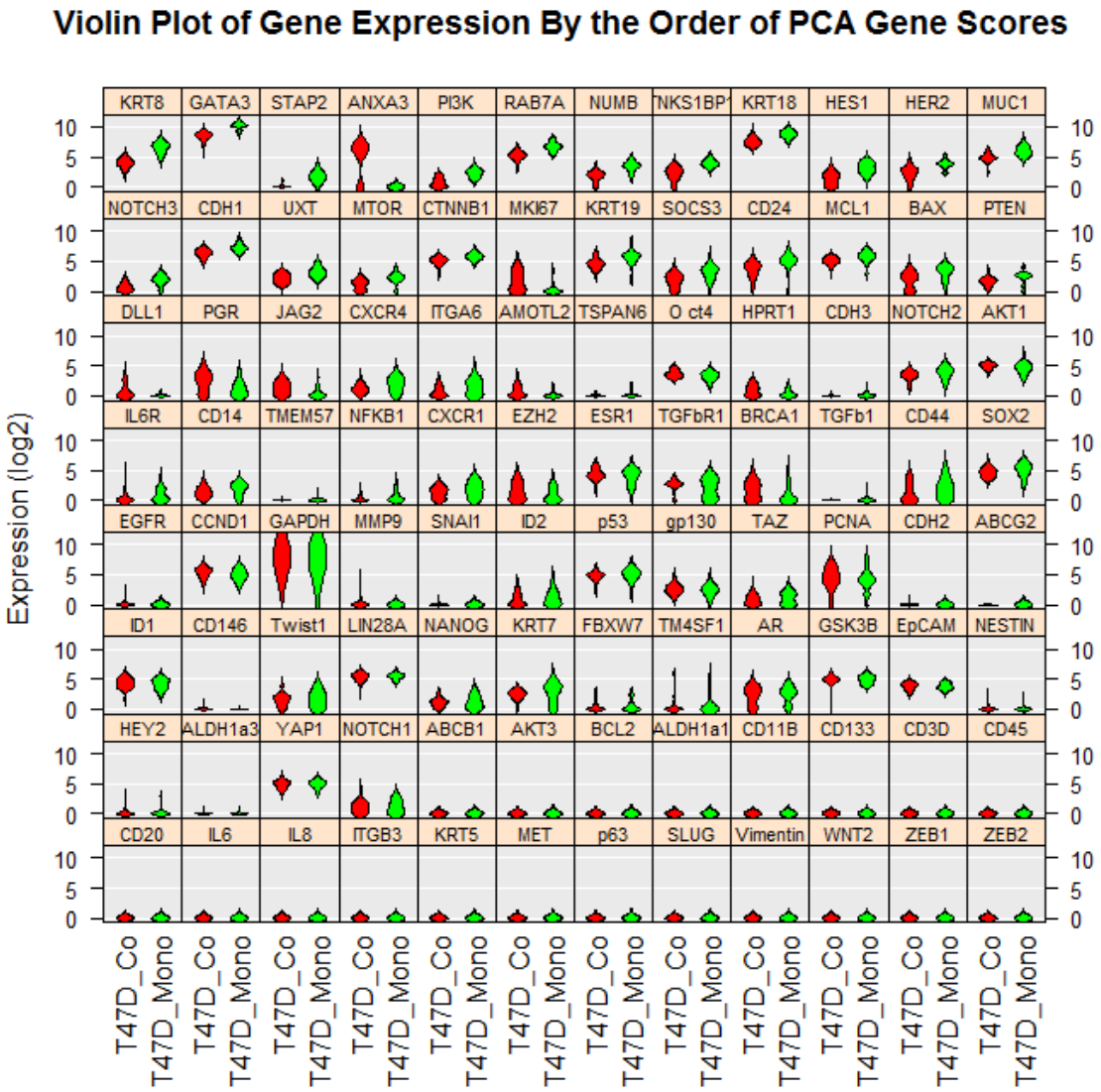
Supplementary Fig. 14. Violin plots of all 96 genes of co-cultured (red, N = 37) and mono-cultured (green, N = 22) cells. The results were not normalized.



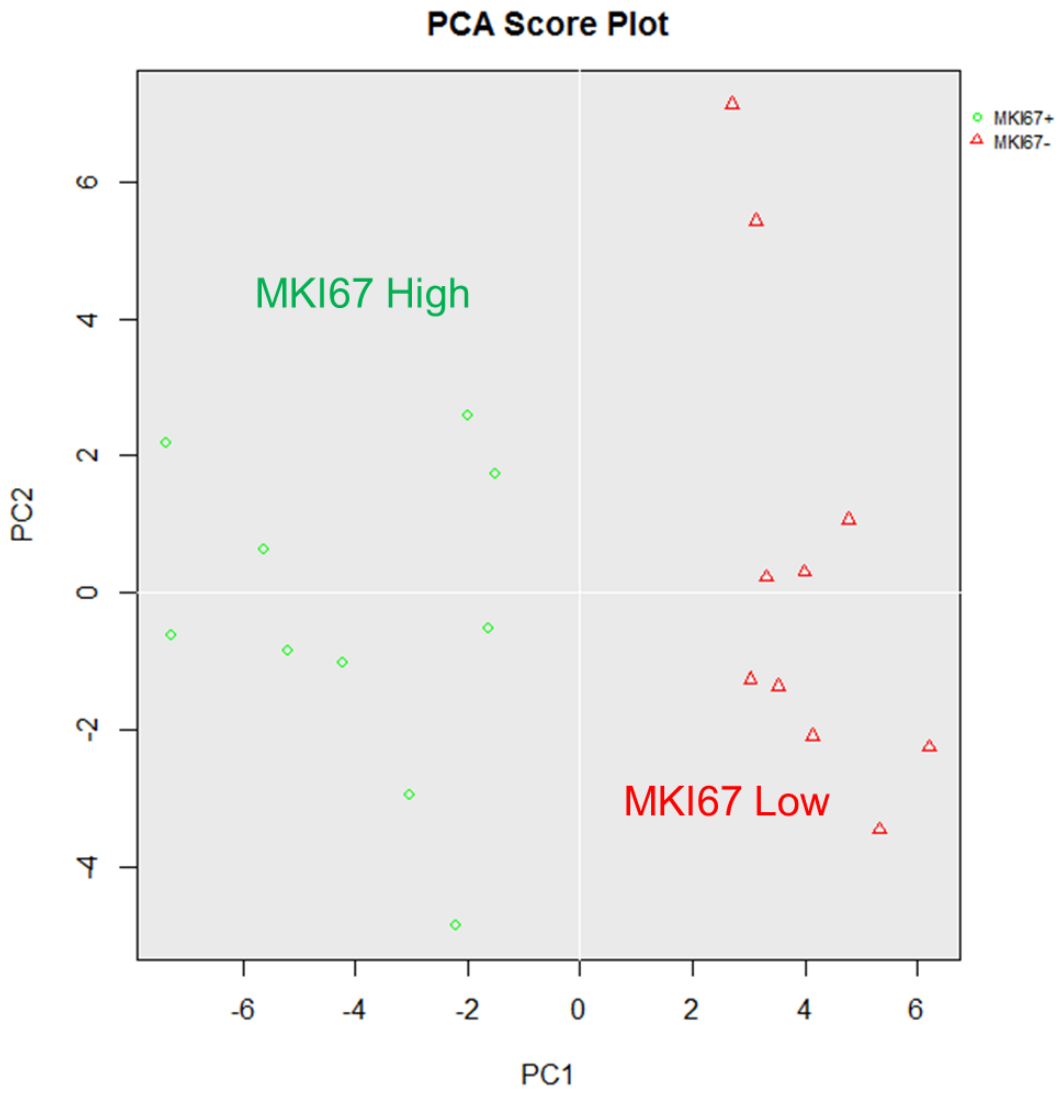
Supplementary Fig. 15: PCA clustering of single cell expression analysis for co-cultured (red, N=37) and mono-cultured (green, N=22) T47D cells compared. The results were normalized by GAPDH.



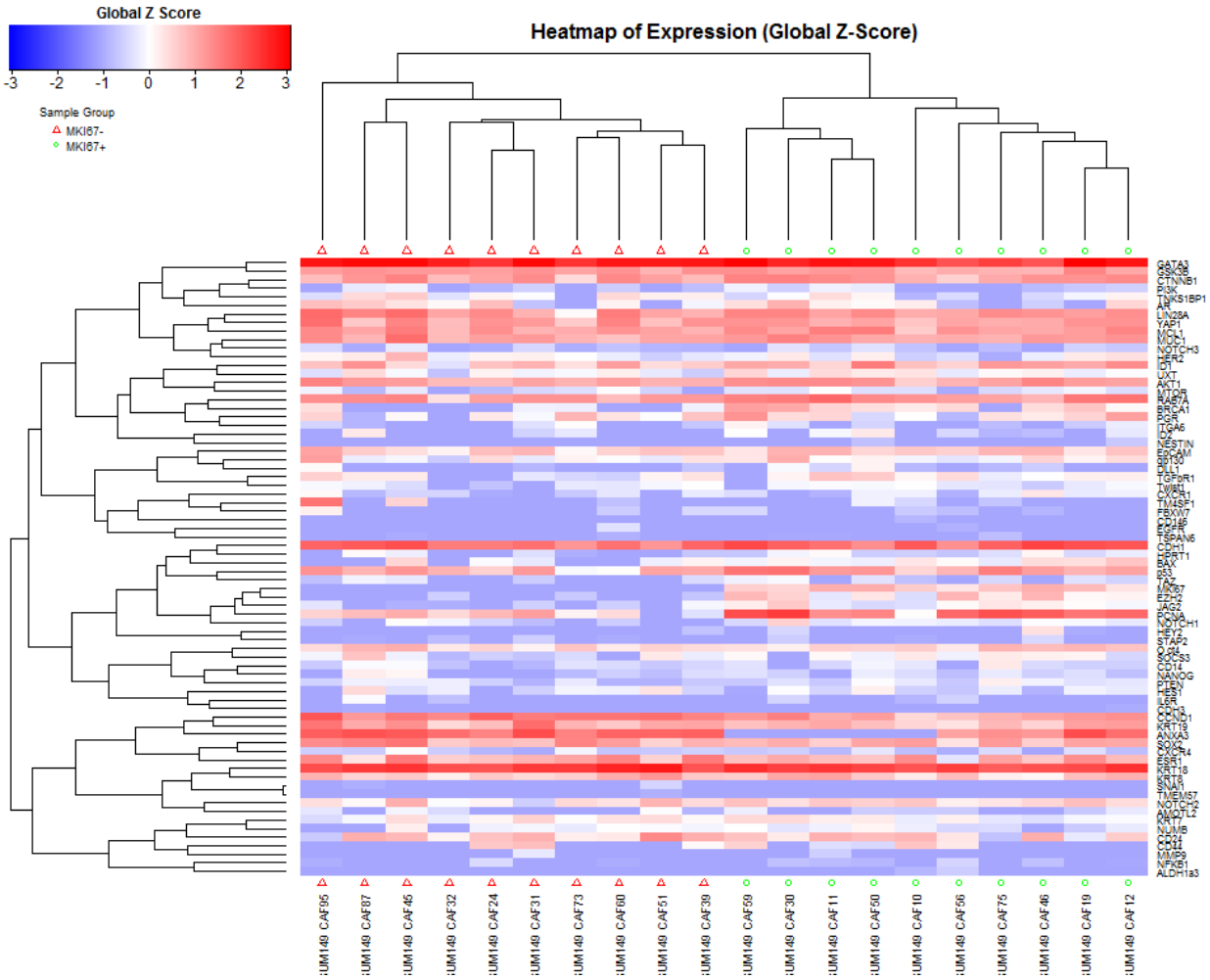
Supplementary Fig. 16. Heatmap hierarchical clustering of single cell expression analysis for between co-cultured (red, N=37) and mono-cultured (green, N=22) T47D cells. The results were normalized by GAPDH.



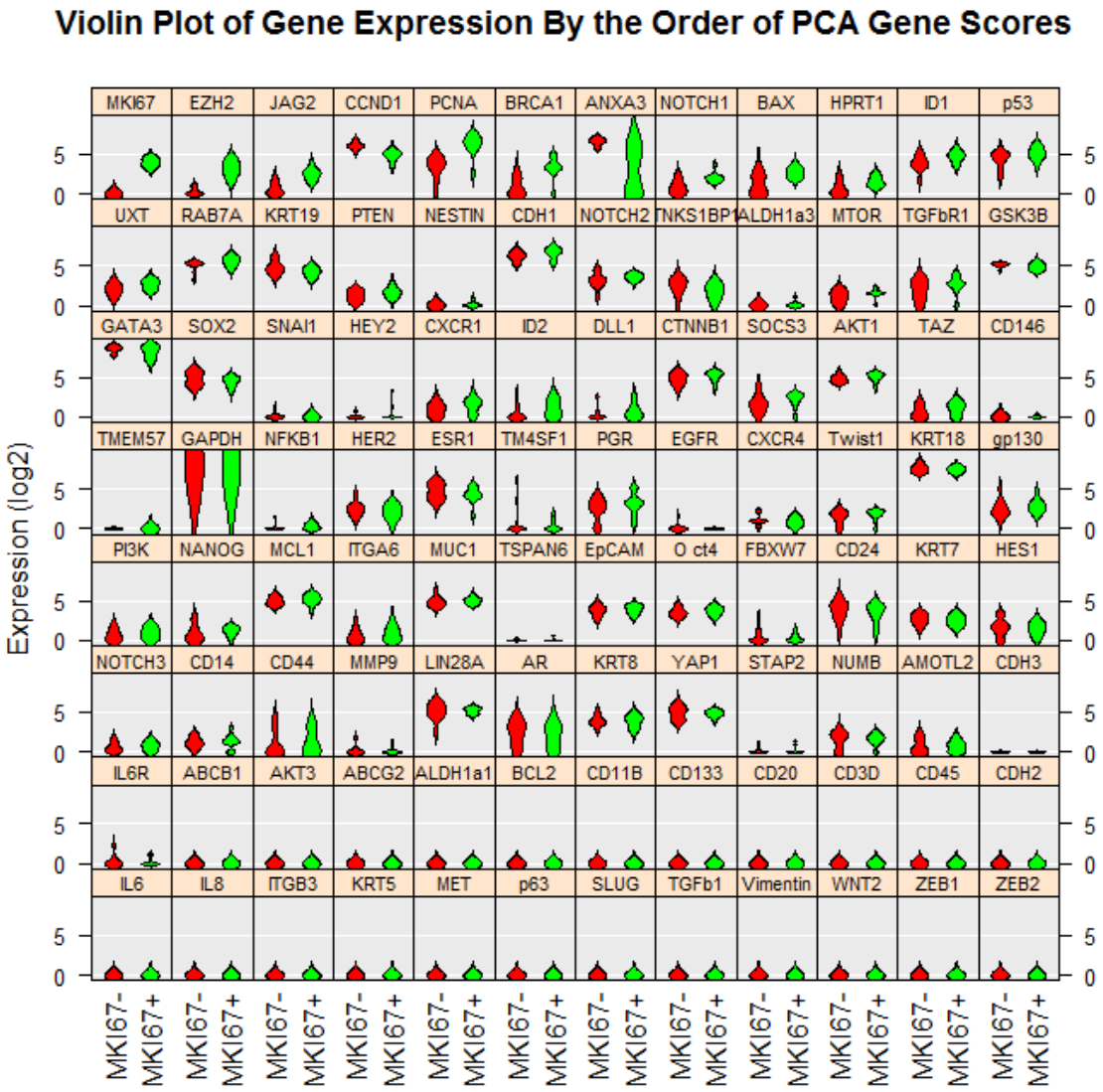
Supplementary Fig. 17. Violin plots of all 96 genes of co-cultured (red, N = 37) and mono-cultured (green, N = 22) cells. The results were normalized by GAPDH.



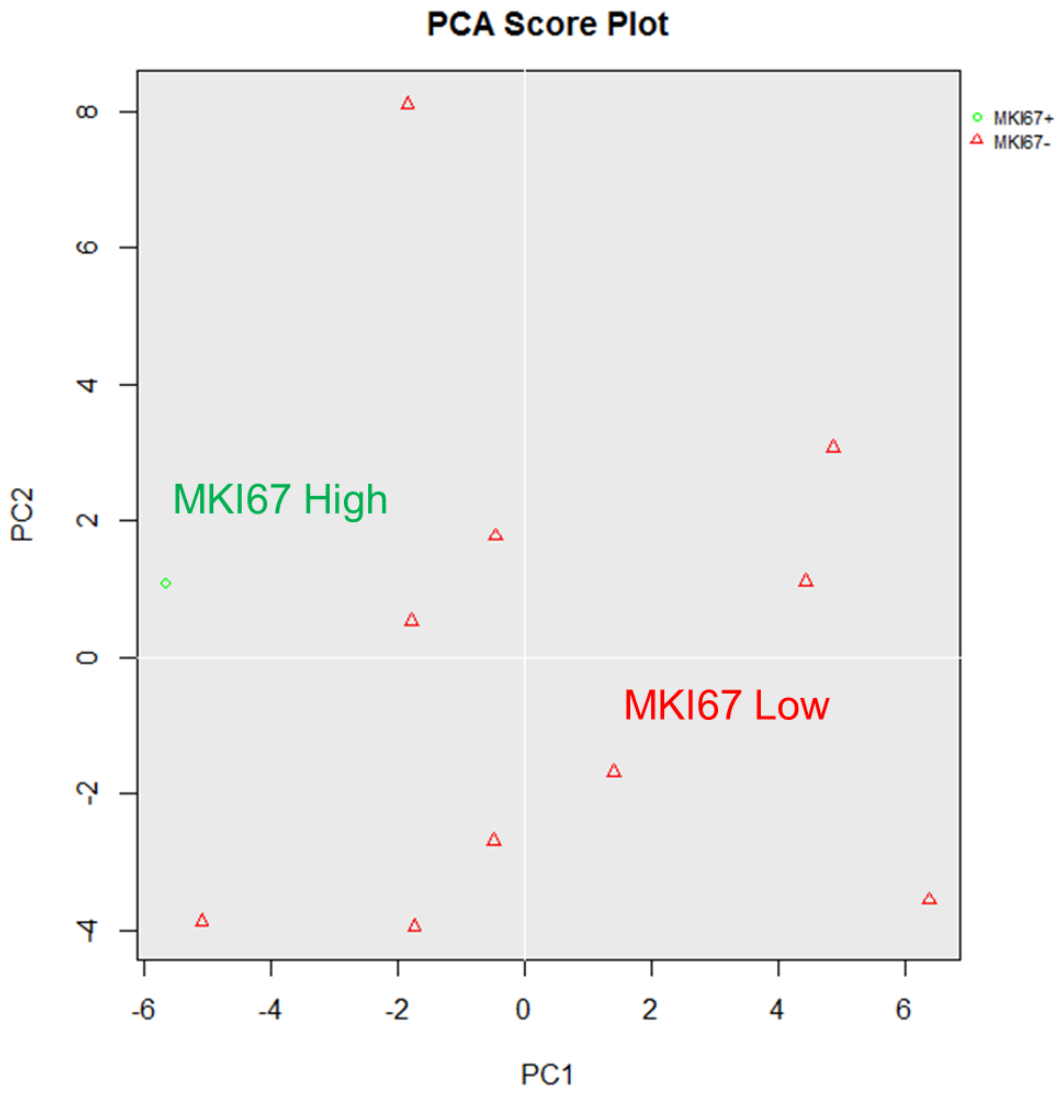
Supplementary Fig. 18: PCA clustering of single cell expression analysis for MKI67 high (green, N=10) and MKI 67 low (red, N=10) cells in the co-cultured group. The results were normalized by GAPDH.



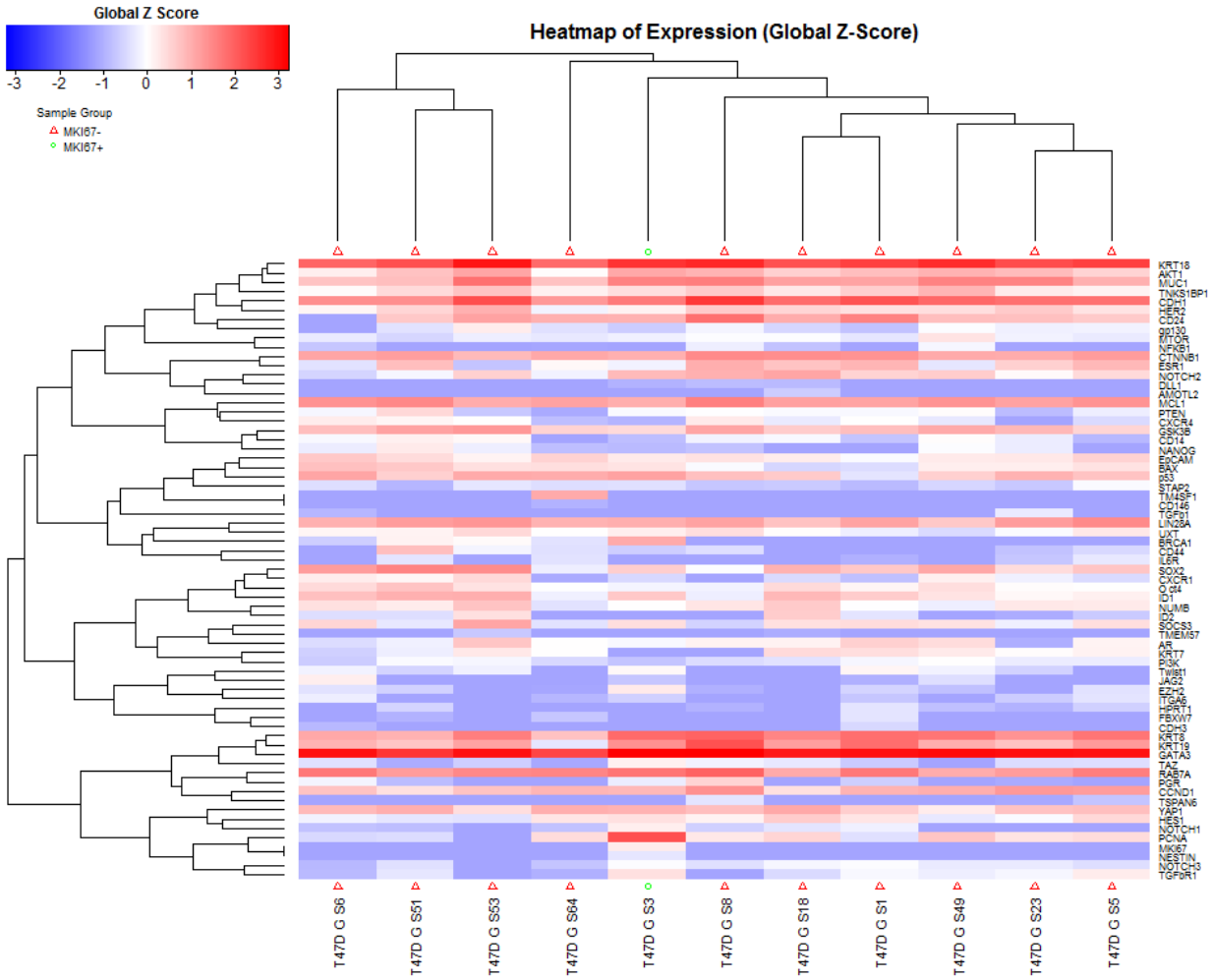
Supplementary Fig. 19. Heatmap hierarchical clustering of single cell expression analysis for MKI67 high (green, N=10) and MKI 67 low (red, N=10) cells in the co-cultured group. The results were normalized by GAPDH.



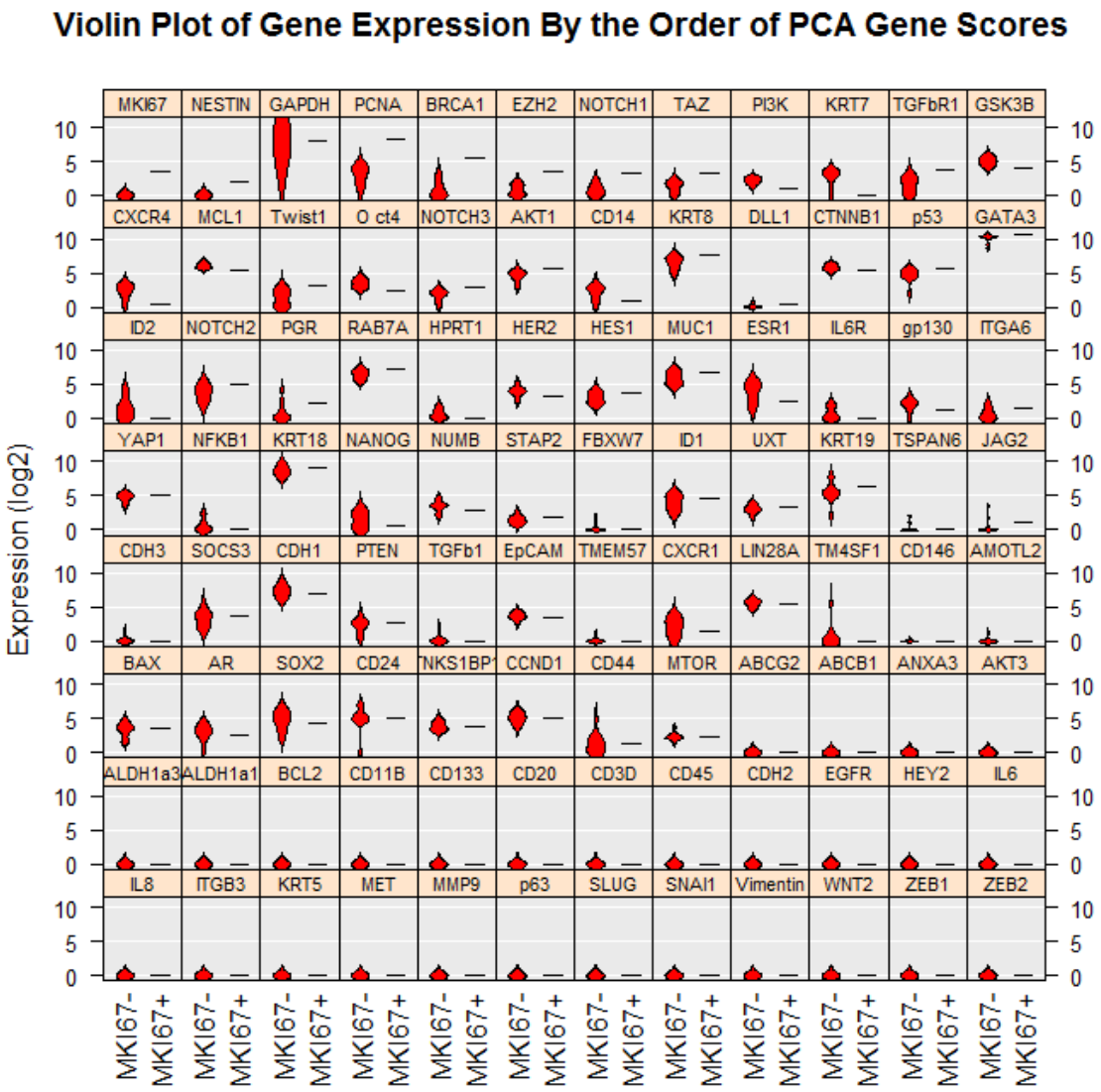
Supplementary Fig. 20. Violin plots of all 96 genes of MKI67 high (green, N=10) and MKI 67 low (red, N=10) cells in the co-cultured group. The results were normalized by GAPDH.



Supplementary Fig. 21: PCA clustering of single cell expression analysis for MKI67 high (green, N=1) and MKI 67 low (red, N=10) cells in the mono-cultured group. The results were normalized by GAPDH.



Supplementary Fig. 22. Heatmap hierarchical clustering of single cell expression analysis for MKI67 high (green, N=1) and MKI 67 low (red, N=10) cells in the mono-cultured group. The results were normalized by GAPDH.



Supplementary Fig. 23. Violin plots of all 96 genes of MKI67 high (green, N=1) and MKI 67 low (red, N=10) cells in the mono-cultured group. The results were normalized by GAPDH.

Reference:

1. Henn A. D.; Rebhahn J.; Brown M. A.; Murphy A. J.; Coca M. N.; Hyrien O.; Pellegrin T.; Mosmann T.; Zand M. S. *J. Immunol.* 2009, **183**, 3177–3187.

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