Supplementary information

A microfluidic platform for profiling of EVs from single breast cancer cells

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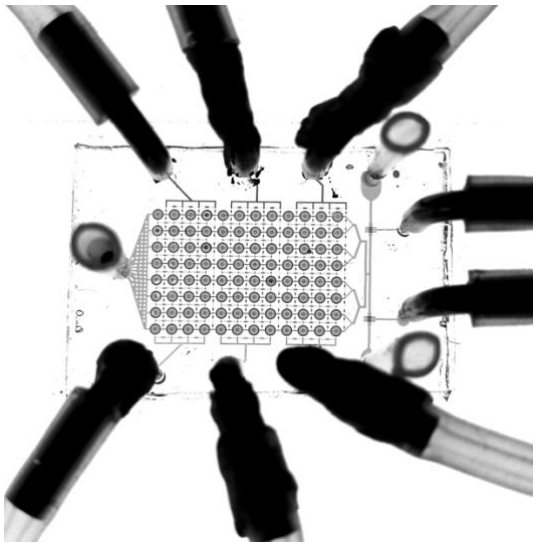

Figure S1. Photograph of the device. The size of the glass slide is 2 x 2.9 cm. Inlets and outlets of the fluid layer are pipette tips, while the pressure layers are connected via tubing to the pressure control system.

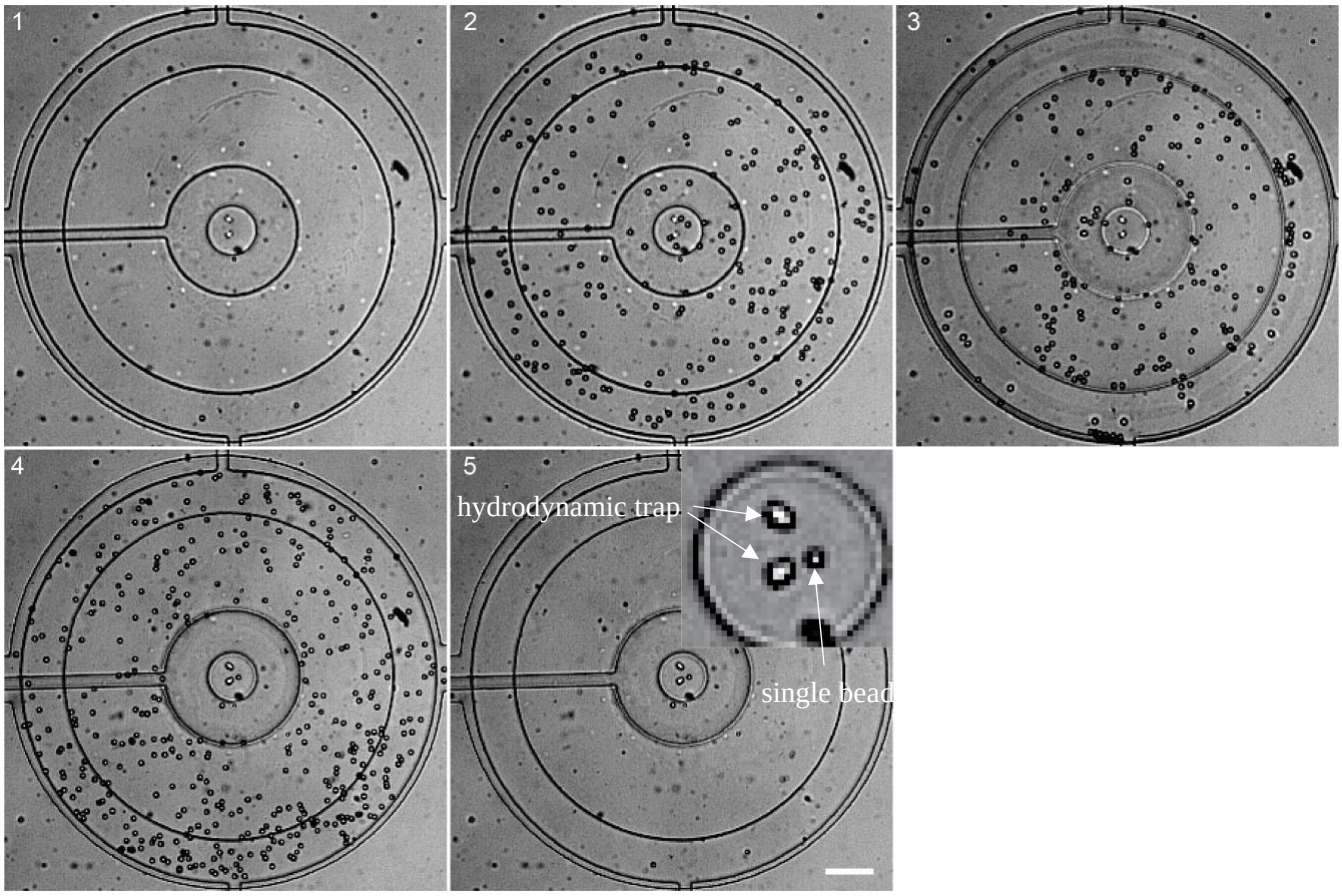

Figure S2. Gap-free spatial isolation and control via separated pneumatic valves in two layers. Using 10 µm beads as a cell surrogate, we tested the device by actuating the valves sequentially, to isolate single cells. Scalebar 50 µm.

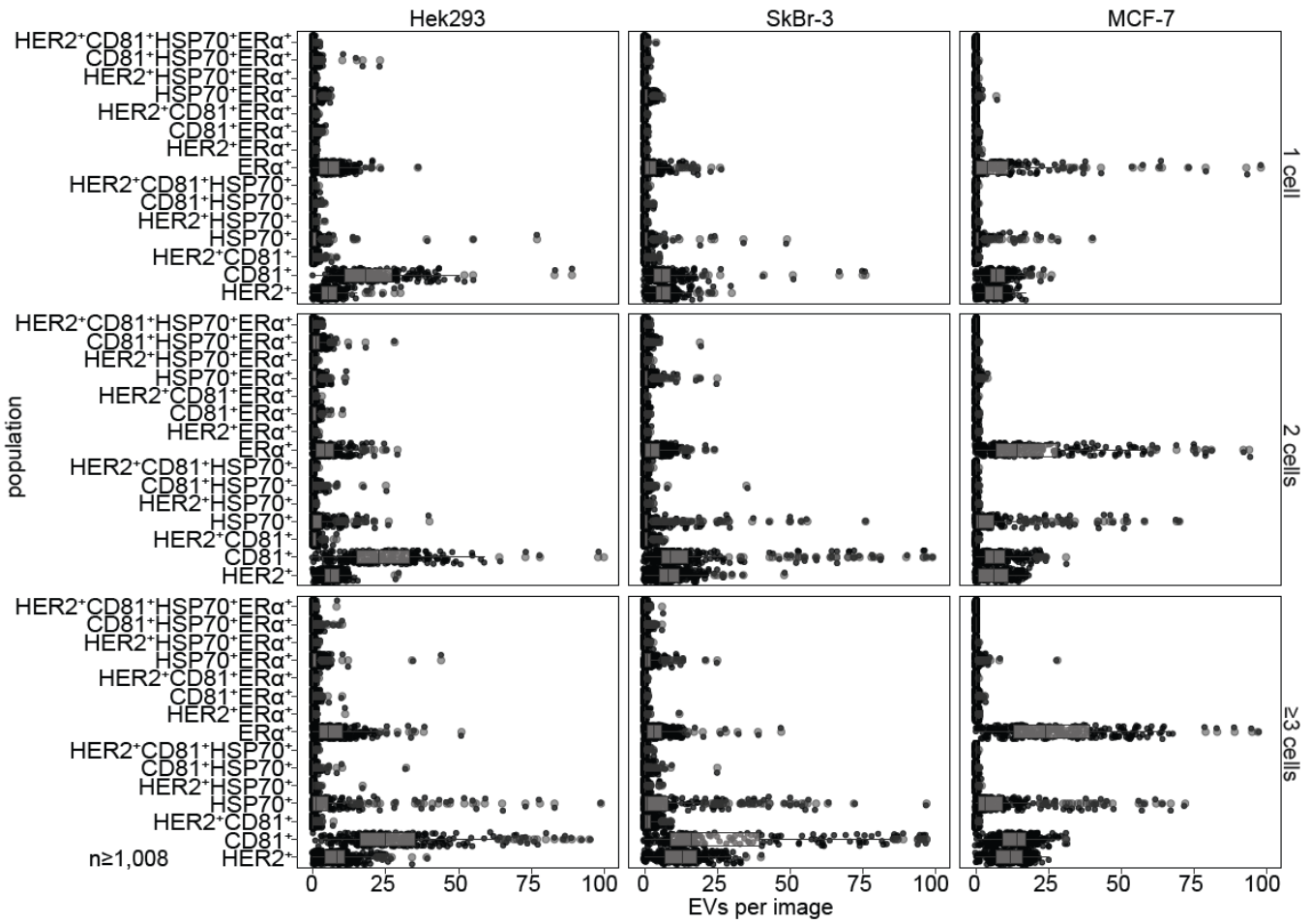

Figure S3. Phenotype analysis of secreted EVs. Dot plots depicting the absolute number of detected EVs, grouped by the cell type and the 15 possible phenotypes, when immobilized on anti-CD63 antibody. The first row depicts the data for 1 cell, the second and third line for two, three and more cells, respectively.

Table SI 1: Statistical test parameters of immobilized EV populations.

Samples	Phenotypes	Parameter 1	Parameter 2	Note
0 cell $_{Hek293}$	not specified	W = 0.77	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
1 cell $_{Hek293}$	not specified	W = 0.60	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
2 cells $_{Hek293}$	not specified	W = 0.73	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
≥3 cells $_{Hek293}$	not specified	W = 0.86	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
0 cell $_{MCF-7}$	not specified	W = 0.73	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
1 cell $_{MCF-7}$	not specified	W = 0.71	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
2 cells $_{MCF-7}$	not specified	W = 0.89	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
≥3 cells $_{MCF-7}$	not specified	W = 0.97	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
0 cell $_{SkBr-3}$	not specified	W = 0.79	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
1 cell $_{SkBr-3}$	not specified	W = 0.49	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
2 cells $_{SkBr-3}$	not specified	W = 0.69	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
≥3 cells $_{SkBr-3}$	not specified	W = 0.89	$p < 2.20 \times 10^{-16}$	Shapiro Wilk
1$_{Hek293}$ vs. 0 cell $_{Hek293}$	not specified	D_{KS} = 0.30	$p < 2.20 \times 10^{-16}$	two-sided KS
2 $_{Hek293}$ vs. 1 cell $_{Hek293}$	not specified	D_{KS} = 0.32	$p < 2.20 \times 10^{-16}$	two-sided KS
≥3 $_{Hek293}$ vs. 2 cells $_{Hek293}$	not specified	D_{KS} = 0.19	$p < 2.20 \times 10^{-16}$	two-sided KS
1 $_{SkBr-3}$ vs. 0 cells $_{SkBr-3}$	not specified	D_{KS} = 0.25	$p < 2.20 \times 10^{-16}$	two-sided KS
2 $_{SkBr-3}$ vs. 1 cell $_{SkBr-3}$	not specified	D_{KS} = 0.31	$p < 2.20 \times 10^{-16}$	two-sided KS
≥3 $_{SkBr-3}$ vs. 2 cells $_{SkBr-3}$	not specified	D_{KS} = 0.42	$p < 2.20 \times 10^{-16}$	two-sided KS
1 $_{MCF-7}$ vs. 0 cells $_{MCF-7}$	not specified	D_{KS} = 0.44	$p < 2.20 \times 10^{-16}$	two-sided KS
2 $_{MCF-7}$ vs. 1 cell $_{MCF-7}$	not specified	D_{KS} = 0.40	$p < 2.20 \times 10^{-16}$	two-sided KS
≥3 $_{MCF-7}$ vs. 2 cells $_{MCF-7}$	not specified	D_{KS} = 0.30	$p < 2.20 \times 10^{-16}$	two-sided KS

2 $cells_{Hek293}$ vs. 2 $cells_{SkBr-3}$	$HER2^+$	D_{KS} = 0.21	p = 1.42 × 10^{-5}	two-sided KS
2 $cells_{SkBr-3}$ vs. 2 $cells_{MCF-7}$	$HER2^+$	D_{KS} = 0.22	p = 1.15 × 10^{-5}	two-sided KS
2 $cells_{Hek293}$ vs. 2 $cells_{MCF-7}$	$HER2^+$	D_{KS} = 0.21	p = 9.32 ×10^{-5}	two-sided KS
≥3 $cells_{Hek293}$ vs. ≥3 $cells_{SkBr-3}$	$HER2^+$	D_{KS} = 0.33	p = 3.64×10^{-13}	two-sided KS
≥3 $cells_{SkBr-3}$ vs. ≥3 $cells_{MCF-7}$	$HER2^+$	D_{KS} = 0.16	p = 3.43 × 10^{-3}	two-sided KS
≥3 $cells_{Hek293}$ vs. ≥3 $cells_{MCF-7}$	$HER2^+$	D_{KS} = 0.28	p = 3.42 × 10^{-9}	two-sided KS
2 $cells_{Hek293}$ vs. 2 $cells_{SkBr-3}$	$HER2^+CD81^+$	D_{KS} = 0.06	p = 0.78	two-sided KS
2 $cells_{SkBr-3}$ vs. 2 $cells_{MCF-7}$	$HER2^+CD81^+$	D_{KS} = 0.24	p = 5.58 × 10^{-7}	two-sided KS
2 $cells_{Hek293}$ vs. 2 $cells_{MCF-7}$	$HER2^+CD81^+$	D_{KS} = 0.224	p = 2.54×10^{-6}	two-sided KS
≥3 $cells_{Hek293}$ vs. ≥3 $cells_{SkBr-3}$	$HER2^+CD81^+$	D_{KS} = 0.38	p < 2.2×10^{-16}	two-sided KS
≥3 $cells_{SkBr-3}$ vs. ≥3 $cells_{MCF-7}$	$HER2^+CD81^+$	D_{KS} = 0.51	p = 2.2 × 10^{-16}	two-sided KS
≥3 $cells_{Hek293}$ vs. ≥3 $cells_{MCF-7}$	$HER2^+CD81^+$	D_{KS} = 0.13	p = 2.51×10^{-2}	two-sided KS
1 $cell_{Hek293}$ vs. 1 $cell_{SkBr-3}$	$ERα^+$	D_{KS} = 0.35	p = 1.02×10^{-11}	two-sided KS
1 $cell_{SkBr-3}$ vs. 1 $cell_{MCF-7}$	$ERα^+$	D_{KS} = 0.29	p = 8.34×10^{-8}	two-sided KS
1 $cells_{Hek293}$ vs. 1$cells_{MCF-7}$	$ERα^+$	D_{KS} = 0.15	p = 1.24×10^{-2}	two-sided KS
2 $cells_{Hek293}$ vs. 2 $cells_{SkBr-3}$	$ERα^+$	D_{KS} = 0.60	p < 2.2 × 10^{-16}	two-sided KS
2 $cells_{SkBr-3}$ vs. 2 $cells_{MCF-7}$	$ERα^+$	D_{KS} = 0.60	p < 2.2 × 10^{-16}	two-sided KS
2 $cells_{Hek293}$ vs. 2 $cells_{MCF-7}$	$ERα^+$	D_{KS} = 0.54	p = 2.2×10^{-6};	two-sided KS
≥3 $cells_{Hek293}$ vs. ≥3 $cells_{SkBr-3}$	$ERα^+$	D_{KS} = 0.38	p < 2.2×10^{-16}	two-sided KS
≥3 $cells_{SkBr-3}$ vs. ≥3 $cells_{MCF-7}$	$ERα^+$	D_{KS} = 0.51	p < 2.2 × 10^{-16}	two-sided KS
≥3 $cells_{Hek293}$ vs. ≥3 $cells_{MCF-7}$	$ERα^+$	D_{KS} = 0.13	p = 2.51×10^{-2}	two-sided KS
1 $cell_{Hek293}$ vs. 1 $cell_{SkBr-3}$	$ERα^+$HSP70	D_{KS} = 0.04	p = 0.99;	two-sided KS
1 $cell_{SkBr-3}$ vs. 1 $cell_{MCF-7}$	$ERα^+$HSP70	D_{KS} = 0.31	p = 1.38×10^{-8}	two-sided KS

1 $cells_{Hek293}$ vs. 1$cells_{MCF-7}$	ERα^{+}HSP70	D_{KS} = 0.26	p = 6.97×10^{-7}	two-sided KS
2 $cells_{Hek293}$ vs. 2 $cells_{SkBr-3}$	ERα^{+}HSP70	D_{KS} = 0.05	p = 0.89	two-sided KS
2 $cells_{SkBr-3}$ vs. 2 $cells_{MCF-7}$	ERα^{+}HSP70	D_{KS} = 0.36	p = 3.78×10^{-15}	two-sided KS
2 $cells_{Hek293}$ vs. 2 $cells_{MCF-7}$	ERα^{+}HSP70	D_{KS} = 0.34	p = 3.69×10^{-12}	two-sided KS
≥3 $cells_{Hek293}$ vs. ≥3 $cells_{SkBr-3}$	ERα^{+}HSP70	D_{KS} = 0.24	p = 3.66×10^{-7}	two-sided KS
≥3 $cells_{SkBr-3}$ vs. ≥3 $cells_{MCF-7}$	ERα^{+}HSP70	D_{KS} = 0.43	p < 2.2×10^{-16}	two-sided KS
≥3 $cells_{Hek293}$ vs. ≥3 $cells_{MCF-7}$	ERα^{+}HSP70	D_{KS} = 0.19	p = 2.24×10^{-4}	two-sided KS
1 $cell_{Hek293}$ vs. 1 $cell_{SkBr-3}$	CD81^{+}ERα^{+}HSP70	D_{KS} = 0.14	p = 2.69×10^{-2}	two-sided KS
1 $cell_{SkBr-3}$ vs. 1 $cell_{MCF-7}$	CD81^{+}ERα^{+}HSP70	D_{KS} = 0.09	p = 0.39	two-sided KS
1 $cells_{Hek293}$ vs. 1$cells_{MCF-7}$	CD81^{+}ERα^{+}HSP70	D_{KS} = 0.23	p = 1.87×10^{-5}	two-sided KS
2 $cells_{Hek293}$ vs. 2 $cells_{SkBr-3}$	CD81^{+}ERα^{+}HSP70	D_{KS} = 0.32	p = 5.67×10^{-12}	two-sided KS
2 $cells_{SkBr-3}$ vs. 2 $cells_{MCF-7}$	CD81^{+}ERα^{+}HSP70	D_{KS} = 0.19	p = 2.3×10^{-4}	two-sided KS
2 $cells_{Hek293}$ vs. 2 $cells_{MCF-7}$	CD81^{+}ERα^{+}HSP70	D_{KS} = 0.51	p < 2.2×10^{-16}	two-sided KS
≥3 $cells_{Hek293}$ vs. ≥3 $cells_{SkBr-3}$	CD81^{+}ERα^{+}HSP70	D_{KS} = 0.04	p = 0.99	two-sided KS
≥3 $cells_{SkBr-3}$ vs. ≥3 $cells_{MCF-7}$	CD81^{+}ERα^{+}HSP70	D_{KS} = 0.23	p = 2.39×10^{-6}	two-sided KS
≥3 $cells_{Hek293}$ vs. ≥3 $cells_{MCF-7}$	CD81^{+}ERα^{+}HSP70	D_{KS} = 0.19	p = 1.54×10^{-4}	two-sided KS
1 $cell_{Hek293}$ vs. 1 $cell_{SkBr-3}$	CD81^{+}	D_{KS} = 0.57	p < 2.2×10^{-16}	two-sided KS
1 $cell_{SkBr-3}$ vs. 1 $cell_{MCF-7}$	CD81^{+}	D_{KS} = 0.17	p = 8.17×10^{-3}	two-sided KS
1 $cells_{Hek293}$ vs. 1$cells_{MCF-7}$	CD81^{+}	D_{KS} = 0.61	p < 2.2×10^{-16}	two-sided KS
2 $cells_{Hek293}$ vs. 2 $cells_{SkBr-3}$	CD81^{+}	D_{KS} = 0.65	p < 2.2×10^{-16}	two-sided KS
2 $cells_{SkBr-3}$ vs. 2 $cells_{MCF-7}$	CD81^{+}	D_{KS} = 0.27	p = 8.08×10^{-8}	two-sided KS
2 $cells_{Hek293}$ vs. 2 $cells_{MCF-7}$	CD81^{+}	D_{KS} = 0.71	p < 2.2×10^{-16}	two-sided KS
≥3 $cells_{Hek293}$ vs. ≥3 $cells_{SkBr-3}$	CD81^{+}	D_{KS} = 0.69	p < 2.2×10^{-16}	two-sided KS

≥3 cells$_{SkBr-3}$ vs. ≥3 cells$_{MCF-7}$	$CD81^+$	$D_{KS} = 0.57$	$p < 2.2\times10^{-16}$	two-sided KS
≥3 cells$_{Hek293}$ vs. ≥3 cells$_{MCF-7}$	$CD81^+$	$D_{KS} = 0.77$	$p < 2.2\times10^{-16}$	two-sided KS
2 cells$_{Hek293}$ vs. 2 cells$_{SkBr-3}$	$CD81^+HSP70^+$	$D_{KS} = 0.08$	$p = 0.39$	two-sided KS
2 cells$_{SkBr-3}$ vs. 2 cells$_{MCF-7}$	$CD81^+HSP70^+$	$D_{KS} = 0.09$	$p = 0.20$	two-sided KS
2 cells$_{Hek293}$ vs. 2 cells$_{MCF-7}$	$CD81^+HSP70^+$	$D_{KS} = 0.17$	$p = 1.96\times10^{-3}$	two-sided KS
≥3 cells$_{Hek293}$ vs. ≥3 cells$_{SkBr-3}$	$CD81^+HSP70^+$	$D_{KS} = 0.08$	$p = 0.43$	two-sided KS
≥3 cells$_{SkBr-3}$ vs. ≥3 cells$_{MCF-7}$	$CD81^+HSP70^+$	$D_{KS} = 0.15$	$p = 6.24\times10^{-3}$	two-sided KS
≥3 cells$_{Hek293}$ vs. ≥3 cells$_{MCF-7}$	$CD81^+HSP70^+$	$D_{KS} = 0.07$	$p = 0.50$	two-sided KS
2 cells$_{Hek293}$ vs. 2 cells$_{SkBr-3}$	$HSP70^+$	$D_{KS} = 0.10$	$p = 0.14$	two-sided KS
2 cells$_{SkBr-3}$ vs. 2 cells$_{MCF-7}$	$HSP70^+$	$D_{KS} = 0.25$	$p = 1.35\times10^{-7}$	two-sided KS
2 cells$_{Hek293}$ vs. 2 cells$_{MCF-7}$	$HSP70^+$	$D_{KS} = 0.17$	$p = 1.96\times10^{-3}$	two-sided KS
≥3 cells$_{Hek293}$ vs. ≥3 cells$_{SkBr-3}$	$HSP70^+$	$D_{KS} = 0.13$	$p = 1.93\times10^{-2}$	two-sided KS
≥3 cells$_{SkBr-3}$ vs. ≥3 cells$_{MCF-7}$	$HSP70^+$	$D_{KS} = 0.18$	$p = 4.65\times10^{-4}$	two-sided KS
≥3 cells$_{Hek293}$ vs. ≥3 cells$_{MCF-7}$	$HSP70^+$	$D_{KS} = 0.31$	$p = 1.74\times10^{-11}$	two-sided KS