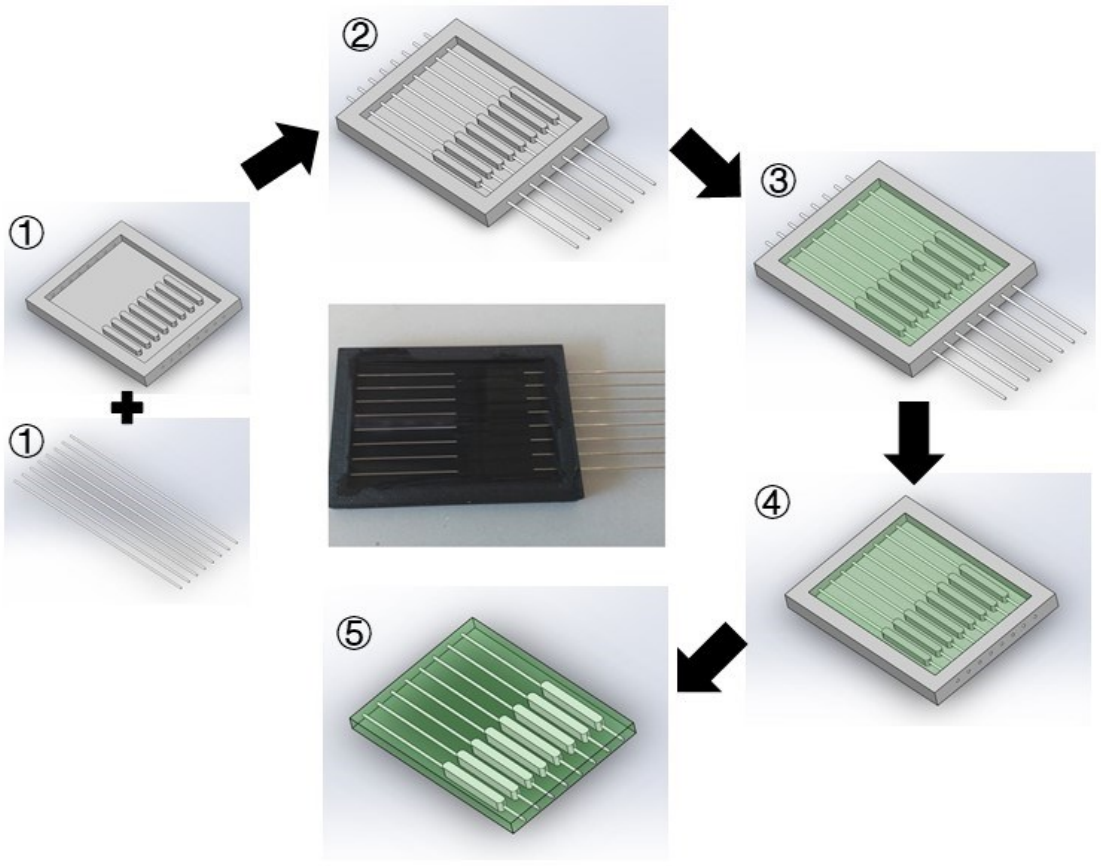
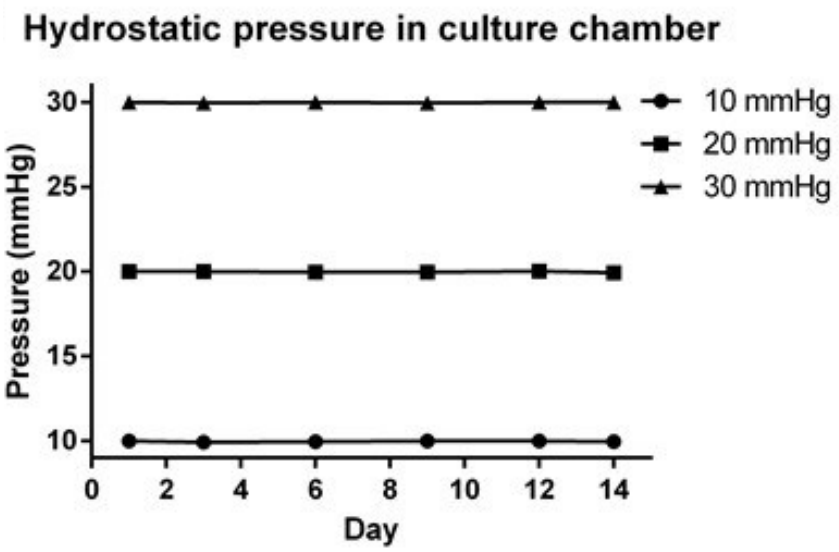
Electronic Supplementary Material (ESI) for Lab on a Chip.

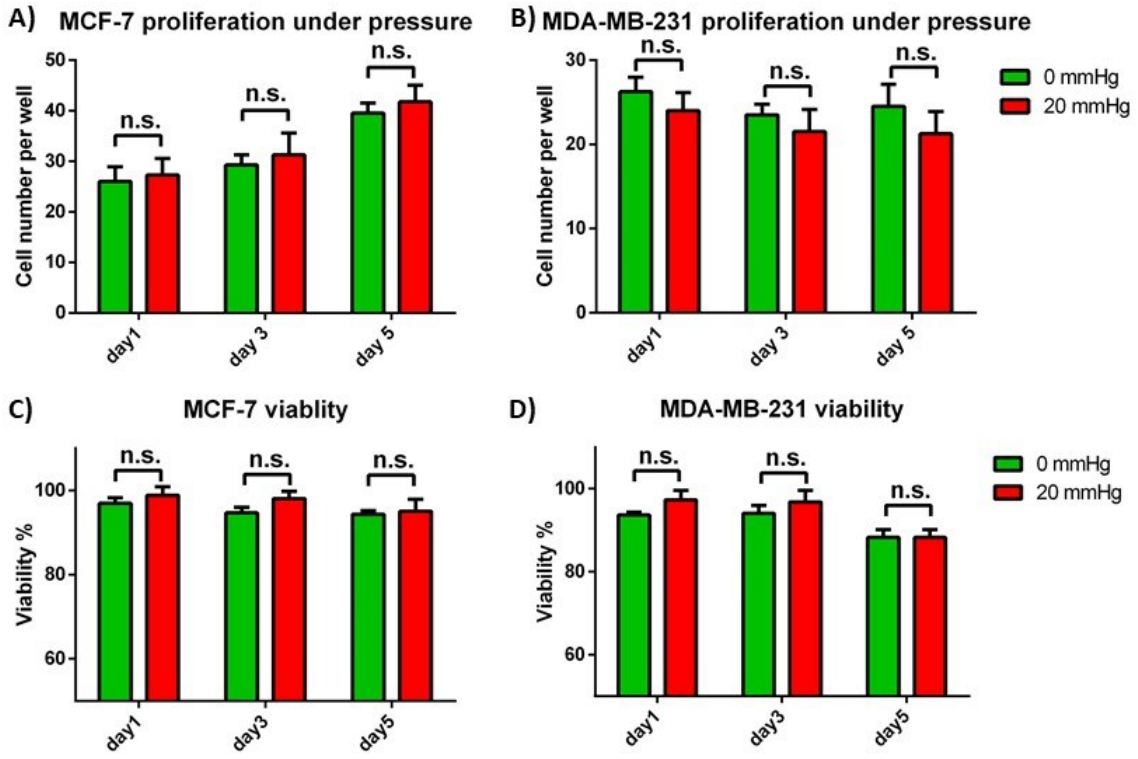
Supplementary Materials



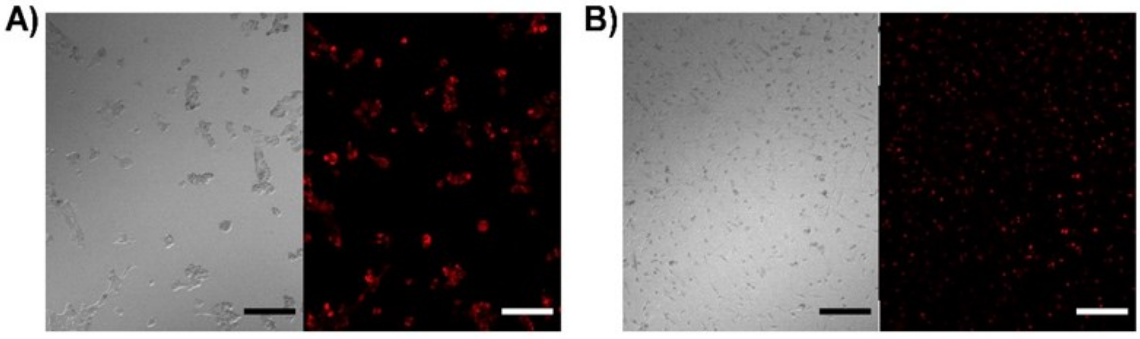
Supplementary Figure 1. Schematic of PDMS barrier fabrication process. A 3D printed mold was fabricated by firstly creating stl. file using SOLIDWOKRS 2016, followed by highest resolution print using flexible photopolymer resin in Formlabs Form 2. The channels in the 3D mold needs to be washed using syringes before curing. The 3D printed mold together with eight stainless steel pins whose diameters are 0.8 mm are used to fabricate the PDMS barrier layer. After insertion of the stainless pins, degassed PDMS prepolymer mixture was poured into the mold and incubated in a warm room (36°C) overnight. After curing the PDMS, the pins were removed, and then the PDMS barrier layer was peeled off from the mold. An actual fabrication picture was shown in the middle of the schematic.



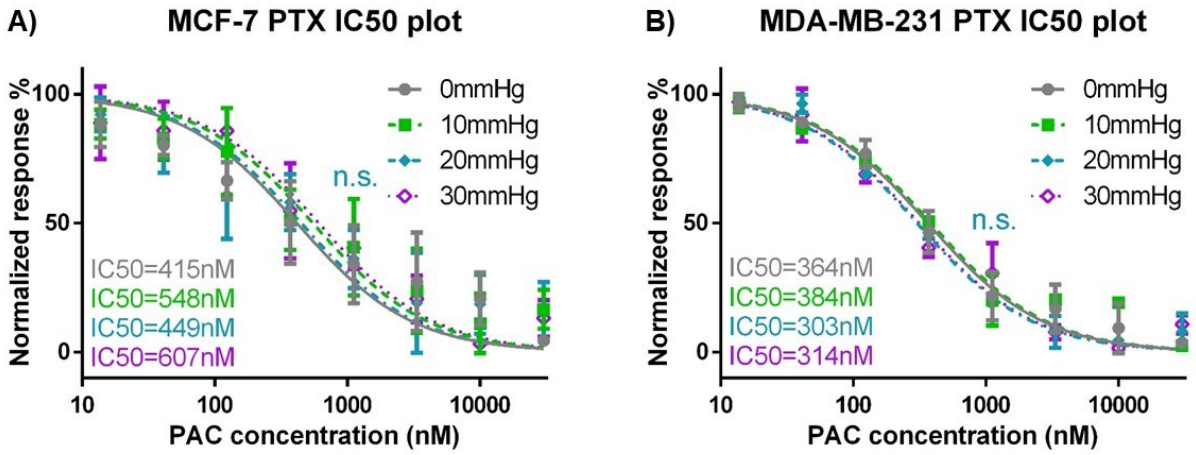
Supplementary Figure 2. Two-week measurement of hydrostatic pressure in the microfluidic chip using VWR manometer. The hydrostatic pressure was very stable and accurate across the two-week measurement with standard deviations of smaller than 0.04. It was within expectation as the culture condition was totally static. The only factor that may affect the pressure was medium evaporation, which was significantly reduced in a humidified incubator. There was no observable change in medium volume within the 3-day refreshing duration. Technical repeats N=3. Mean ± SD.



Supplementary Figure 3. Phenotyping of spheroids under different pressures. A) & B) Representative images of Hoechst, Calcein-AM, and PI staining of spheroids formed by MCF-7 and MDA-MB-231. C)&D) Proliferation of MCF-7 and MDA-MB-231 cells in microwells. Green bars represent 0 mmHg condition; Red bar represents 20 mmHg condition. n.s is $p>0.05$.



Supplementary Figure 4. Transfection efficiency of siRNA. A) & B) Representative bright field and fluorescent images of BLOCK-iT™ Alexa Fluor™ Red Fluorescent Control in MCF-7 and MDA-MB-231. Scale bar = 100 μm.



Supplementary Figure 5. Paclitaxel (PTX) testing under different pressure conditions in MCF-7 and MDA-MB-231 cells. A) & B) PTX IC50 curve of MCF-7 and MDA-MB-231 cells under 0, 10, 20 ,30 mmHg conditions. The result of the two-way ANOVA test between 0 and 20 mmHg at 1 μM PTX is indicated in the figures. No significant difference was observed between all pressure conditions in either cell type. n=3.

Supplementary Table 1. Cluster formation in 39 liquid biopsy samples from breast cancer patients. In both metastatic status and presence of clusters, 'Y' stands for 'Yes' and 'N' stands for 'No.'

ID	Hormone receptor status	Timepoint	Metastatic status	Remarks	Presence of clusters
BC277	ER- PR- HER2+	Post-treatment	N	5-FU testing	Y
BC255	ER+PR+HER2-	Post-treatment	Y	Dox testing	Y
BC266	ER+PR+HER2-	Post-treatment	Y	Negative cluster formation	N
BC262	ER+PR+HER2-	Pre-treatment	N	Dox testing	Y
BC286	ER+PR+ HER2-	Post-treatment	Y	Dox testing	Y
BC271	ER+PR+ HER2+	Pre-treatment	N	Negative cluster formation	N
BC287	ER+PR- HER2-	Post-treatment	N	device characterization	Y
BC280	ER+PR+ HER2-	Post-treatment	Y	Negative cluster formation	Y
BC283	ER+ PR- HER2-	Post-treatment	N	Dox testing	Y
BC261	ER+PR- HER2+	Post-treatment	N	Negative cluster formation	N
BC277	ER- PR- HER2+	Pre-treatment	N	cell line development	Y
BC287	ER+PR- HER2-	Pre-treatment	N	Negative cluster formation	N
BC247	ER+PR+ HER2-	Post-treatment	Y	5-FU testing	Y
BC130	ER-PR- HER2+	Post-treatment	Y	Contamination	N
BC271	ER+PR+ HER2+	Post-treatment	N	Negative cluster formation	N

BC263	ER+PR+ HER2-	Post-treatment	Y	Contamination	N
BC323	ER+PR- HER2-	Pre-treatment	N	Contamination	N
BC325	ER+PR+ HER2-	Pre-treatment	Y	Negative cluster formation	N
BC345	ER-PR+ HER2+	Pre-treatment	Y	Contamination	N
BC347	ER+PR- HER2-	Pre-treatment	Y	Negative cluster formation	N
BC256		Pre-treatment	Y	PCR	Y
BC325	ER+PR+ HER2-	Pre-treatment	Y	Dox testing	Y
BC343	ER+PR+ HER2+	Post-treatment	Y	Negative cluster formation	N
BC352	ER+PR+ HER2+	Pre-treatment	Y	Dox testing	Y
BC347	ER+PR+ HER2-	Post-treatment	Y	CTC subpopulation immunostaining	Y
BC359	ER-PR- HER2+	Pre-treatment	N	Dox and 5FU testing	Y
BC362	ER+PR+ HER2-	Post-treatment	Y	Dox testing	Y
BC366	ER+PR+ HER2-	Pre-treatment	N	PTX testing	Y
BC367	ER+PR+ HER2+	Pre-treatment	N	Dox testing	Y
BC359	ER+PR- HER2+	Pre-treatment	N	Negative cluster formation	N
BC373	ER+PR+ HER2-	Pre-treatment	Y	Dox testing	Y
BC374	ER+PR- HER2-	Pre-treatment	Y	Dox testing	Y
BC373	ER+PR+ HER2-	Pre-treatment	Y	Dox testing	Y

BC376	ER+PR+ HER2+	Pre-treatment	N	Negative cluster formation	N
BC378	ER+PR- HER2-	Pre-treatment	Y	DOX testing	Y
BC379	ER-PR- HER2+	Pre-treatment	Y	PTX testing	Y
BC366	ER+PR- HER2-	Post-treatment	N	cell line development	Y
BC380	ER+PR+ HER2-	Pre-treatment	Y	Negative cluster formation	N
BC378	ER+ PR- HER2-	Post-treatment	Y	PTX testing	Y