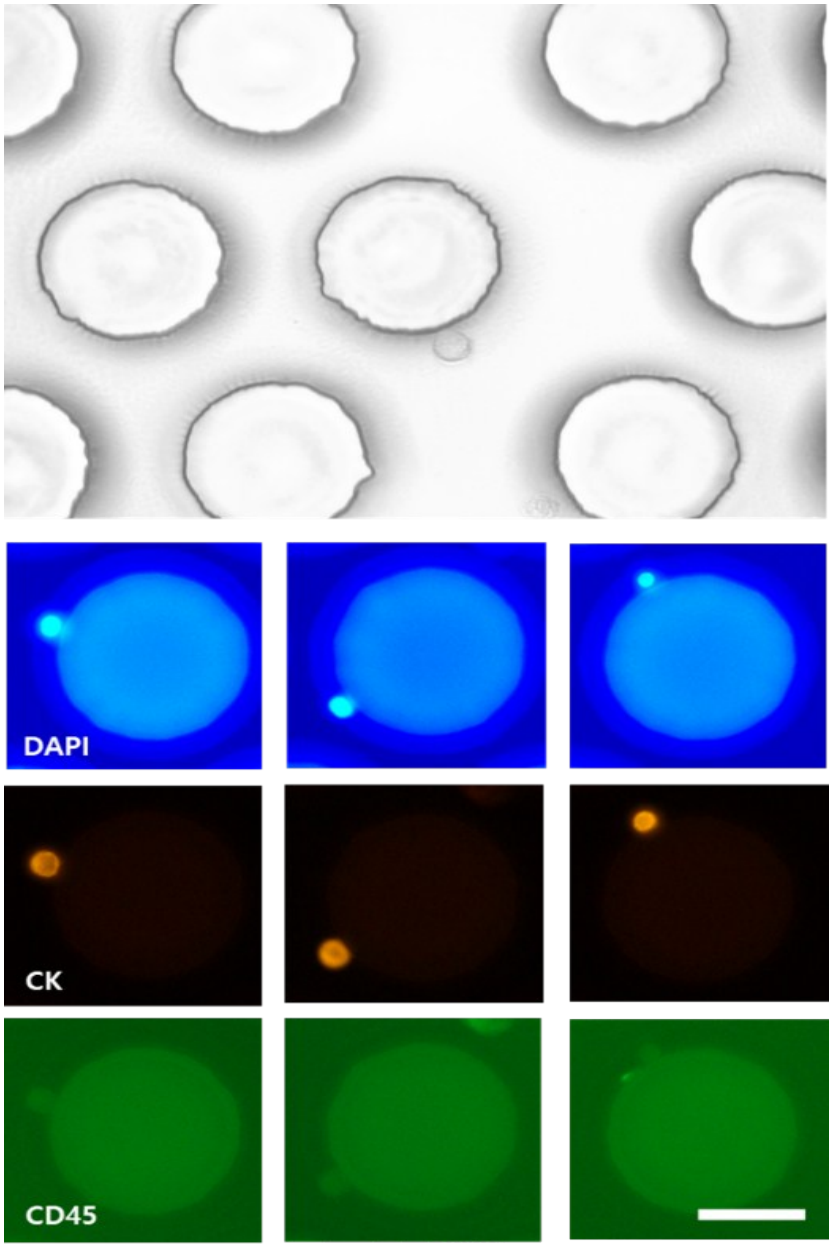
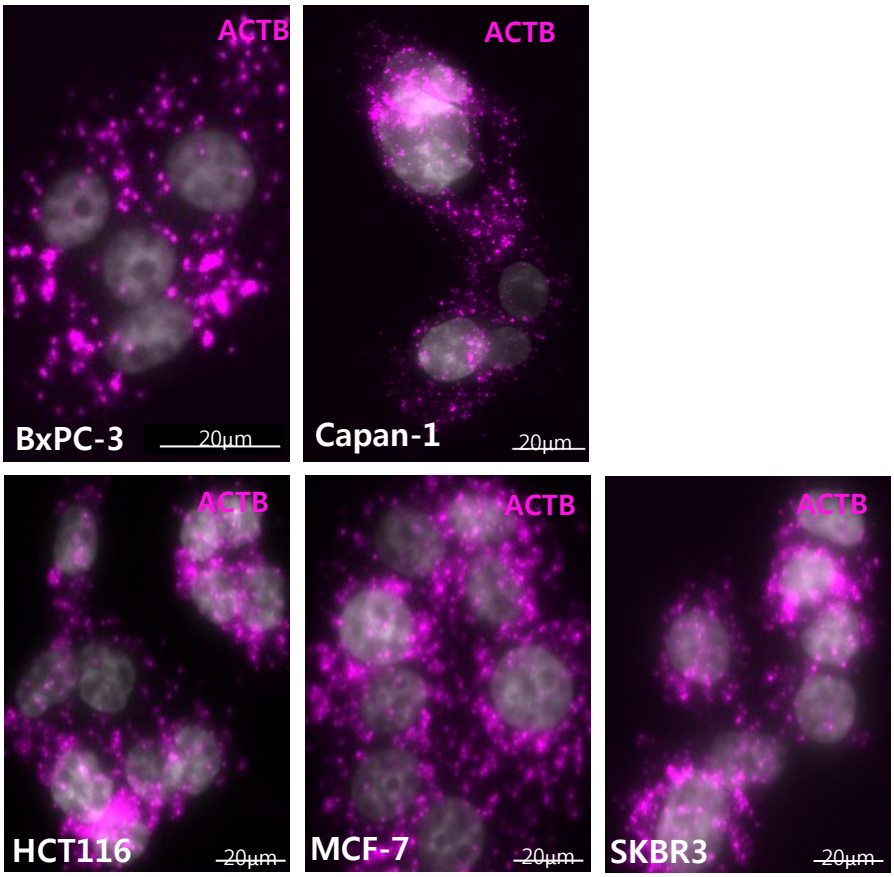
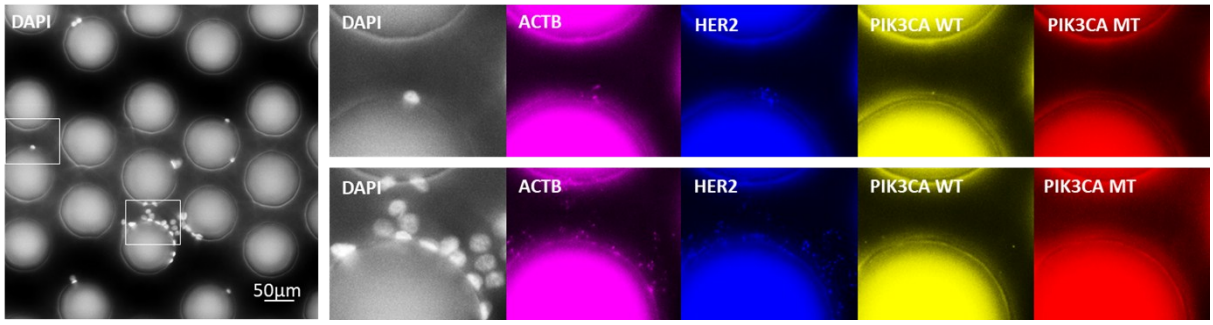
Electronic Supplementary Material (ESI) for Lab on a Chip.



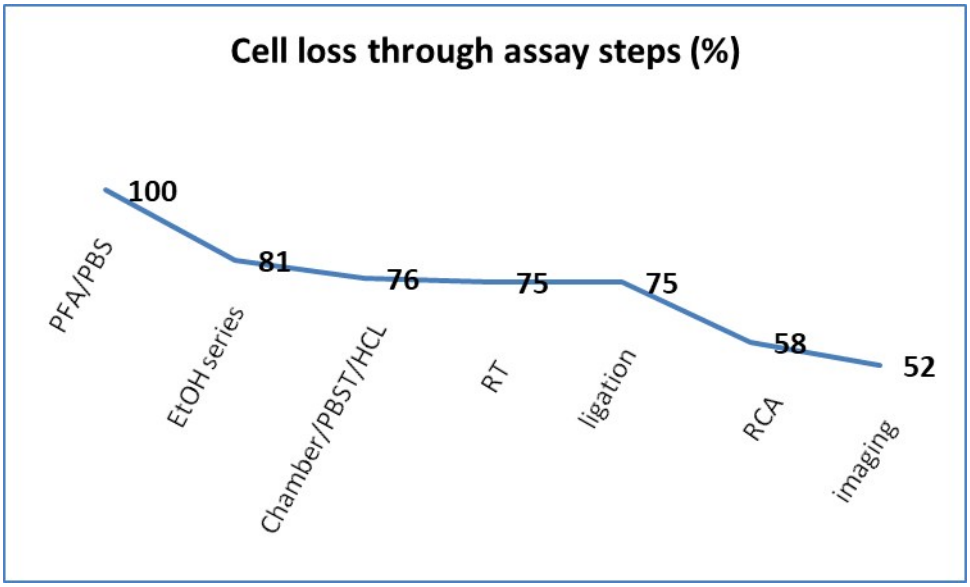
Supplementary Fig 1: SKBR3 cells were spiked in whole blood, run through the biochip and immunostained to measure the capture efficiency of the EpCAM positive cells, which was approximately 98.7%. After capture of cells, DAPI was used for nuclear staining of the cells, cytokeratin to validate their cancerous origin and CD45 was used to stain the white blood cells. Scale bar, 50 μm.



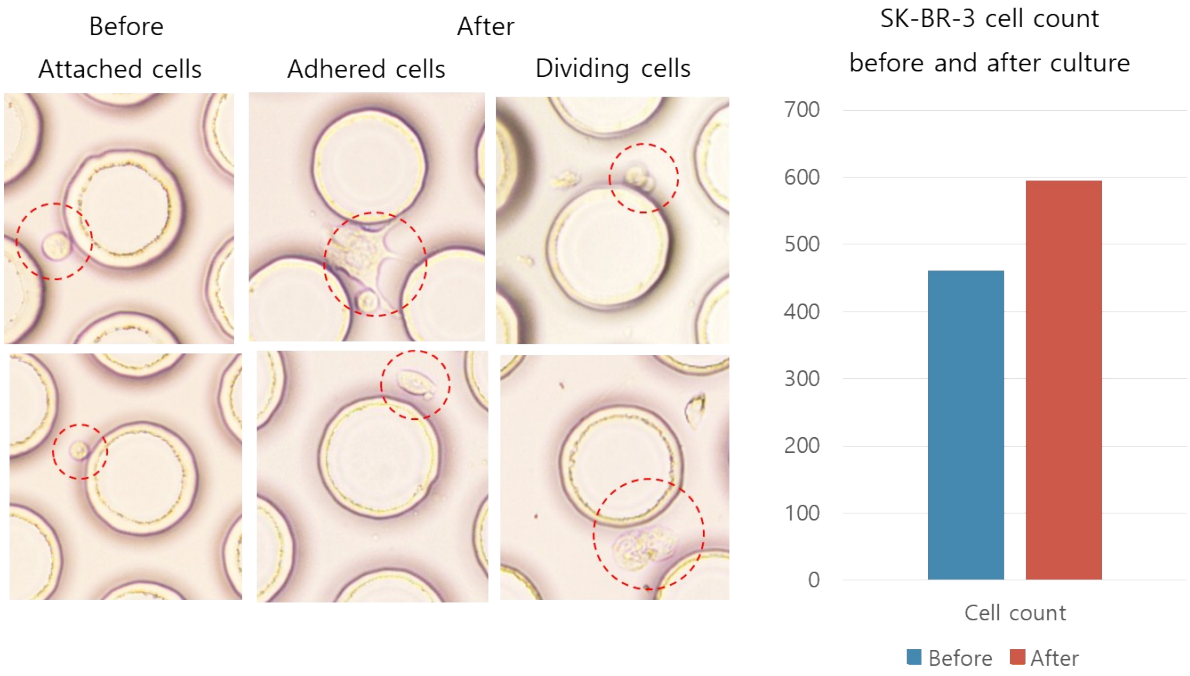
Supplementary Fig 2: Expression of ACTB in different cell lines used for the study. As a representative housekeeping gene, ACTB expressions serve as an internal assay control for normalizing other gene expressions.



Supplementary Fig 3: *In situ* molecular characterization of SKBR3 cell lines on chip. After the cells were spiked into the whole blood, analysis with four different probes was performed: *ACTB*, HER2 (*ERBB2*), *PIK3CA* wildtype (WT), and *PIK3CA* mutant (MT).



Supplementary Fig 4: Loss in cell number during each step of the assay, expressed as % values, as tested on SKBR3 cells loaded on-chip.



Supplementary Fig 5 The captured epithelial cells can be cultured on chip despite of their attachment to the anti-EpCAM antibodies. The SK-BR-3 cells were attached to the anti-EpCAM antibody-coated pillars and were incubated for 52 hours. The cell morphologies showing their adhesion to the surface of the chip and their division show that they can be cultured on OPENchip (Left pictures). The cells were counted before (blue) and after (red) the incubation (Right graph).

Supplementary Table 1

Cancer type	Molecule type	Biomarker	Test detects	When	Clinical actions	Evidence	Solid tumor therapy matches
Invasive breast cancer stage I-IV	RNA	HER2 (ERBB) gene	Gene amplification	Invasive, early stage, metastatic	Predictor of response to HER2-targeted therapy such as trastuzumab, pertuzumab, lapatinib, or trastuzumab emtansine	El-Deiry et al. CA Cancer J Clin, 2019	Trastuzumab emtansine (NCI-MATCH trial) [a] Trastuzumab and pertuzumab (TAPUR trial) [b]
Invasive breast cancer stage I-IV	DNA	PIK3CA gene	Single nucleotide mutation	Invasive, early stage, metastatic	Predictor of progression-free survival rate (PFS). If the nucleotide is not mutated, the PFS is approximated to be 9 years, while PFS is 4 years if the nucleotide is mutated.	Li et al. Am J Cancer Res 2018	Taselisib and Copanlisib (NCI-MATCH trial) [a]
Pancreatic Cancer	DNA	KRAS gene	Single nucleotide mutation	Invasive, early stage, metastatic	Prognostic factor of pancreatic cancer (95% prevalence)	Bryant et al. Trends Biochem Sci 2014	Cetuximab when wild type[a]

[a] NCI-MATCH Trial: Targeted therapy directed by genetic testing in treating patients with advanced refractory solid tumors, lymphomas, or multiple myeloma. Matches are as listed on clinicaltrials.gov/ct2show/NCT02565060.
[b] The American Society of Clinical Oncology's TAPUR trial: Testing the use of US Food and Drug Administration-approved drugs that target a specific abnormality in a tumor gene in people with advanced stage cancer. Matches are as listed on clinical trials.gov/ct2/show/NCT02693535
Table adopted and modified from El-Deiry et al. CA Cancer J Clin, 2019.

Supplementary Table 2

Padlock probes	
Pancreas	
Pd_KRAS_G12_wt	/5Phos/GTGGCGTAGGCAAGATCCTAGTAATCAGTAGCCGTGACTATCGACT GGTTCAAAGTGGTAGTTGGAGCTG
Pd_KRAS_G12S	/5Phos/GTGGCGTAGGCAAGATTCTAGATCCCTCAATGCACATGTTTGGCTC CGGTTCAAGTGGTAGTTGGAGCTA
Pd_KRAS_G12R	/5Phos/GTGGCGTAGGCAAGATTCTAGATCCCTCAATGCACATGTTTGGCTC CGGTTCAAGTGGTAGTTGGAGCTC
Pd_KRAS_G12C	/5Phos/GTGGCGTAGGCAAGATTCTAGATCCCTCAATGCACATGTTTGGCTC CGGTTCAAGTGGTAGTTGGAGCTT
Pd_KRAS_G12A	/5Phos/TGGCGTAGGCAAGAGTTCTAGATCCCTCAATGCACATGTTTGGCTC CGGTTCAAGGGTAGTTGGAGCTGC
Pd_KRAS_G12V	/5Phos/TGGCGTAGGCAAGAGTTCTAGATCCCTCAATGCACATGTTTGGCTC CGGTTCAAGGGTAGTTGGAGCTGT
Pd_KRAS_G13D	/5Phos/CGTAGGCAAGAGTGCTTCTAGATCCCTCAATGCACATGTTTGGCTC CGGTTCAAGAGTTGGAGCTGGTGA
Pd_ACTB	/5Phos/AGCCTCGCCTTTGCCTTCCTTTTACGACCTCAATGCTGCTGCTGTAC TACTCTTCGCCCCGCGAGCACAG
Breast	
Pd_HER2_1	/5Phos/AACAAAAGCGACCCATTCATCCTCTATGATTACTGACTGCGTCTATT TAGTGGAGCCGCGCCTATCTTTCCTAAGGGAGTGTCTAAG
Pd_HER2_2	/5Phos/GCCCTGCACCTCCTGGATATTCCTCTATGATTACTGACTGCGTCTAT TTAGTGGAGCCAGGACTATCTTCTTTTTGTGAGCGATGAGCACGTA
Pd_PIK3CA_E545_wt	/5phos/AGCAGGAGAAAGATTTTCTACCTTTCCTTTTACGACCGAGATGTACC GCTATCGTTCTTCTATCTTCTTTTCCTCTCTCTGAAATCACTG
Pd_PIK3CA_E545K	/5phos/AGCAGGAGAAAGATTTTCTACCTTTCCTTTTACGAAGTCGGAAGTA CTACTCTCTTCTTCTATCTTCTTTTCCTCTCTCTGAAATCACTA
Pd_PIK3CA_H1047_wt	/5Phos/TCATGGTGGCTGGAC CTAGTTC CCGAGATGTACCGCTATCGT AGTCAGA ACAAATGAATGATGCACA
Pd_PIK3CA_H1047R	/5Phos/TCATGGTGGCTGGAC CTTCTTA AGTCGGAAGTACTACTCTCT CTCTAAA CAAATGAATGATGCACG
Pd_PIK3CA_H1047L	/5Phos/TCATGGTGGCTGGAC CTTCTTA AGTCGGAAGTACTACTCTCT CTCTAAA CAAATGAATGATGCACT
Pd_ACTB	/5Phos/AGCCTCGCCTTTGCCTTCCTTTTACGACCTCAATGCTGCTGCTGTAC TACTCTTCGCCCCGCGAGCACAG

cDNA primers	
Pancreas	
Primer_KRAS	CC+TC+TA+TT+GT+TGG+AT+CATATTCGTC
Primer_ACTB	C+GG+GC+GG+CG+GATCGGCAAAG
Breast	
Primer_HER2_1	TGAATGGGTCGCTTTTGTT
Primer_HER2_2	ATATCCAGGAGGTGCAGGGC
Primer_PIK3CA_545	+C+AA+TA+GT+GT+CTGTGACTCCATAGAAAA
Primer_PIK3CA_1047	+GA+AGA+TC+CA+ATCCATTTTTGTTGT
Primer_ACTB	C+GG+GC+GG+CG+GATCGGCAAAG
Detection oligos	
Pancreas	
DO_KRAS_wt	AGTAGCCGTGACTATCGACT
DO_KRAS_mut	CCTCAATGCACATGTTTGGCTCC
DO_ACTB	CCTCAATGCTGCTGCTGTACTAC
Breast	
DO_HER2	TGCGTCTATTTAGTGGAGCC
DO_PIK3CA_wt	CCGAGATGTACCGCTATCGT
DO_PIK3CA_mut	AGTCGGAAGTACTACTCTCT
DO_ACTB	CCTCAATGCTGCTGCTGTACTAC

Supplementary Note 1

Patient clinical information

For the breast cancer CTC sample, the blood sample used was obtained in February, 2018, from a metastatic breast cancer patient showing disease progression on multiple lines of therapy. The patient was initially diagnosed with clinical stage II (cT2N1) invasive ductal carcinoma at age 44 in July, 2013. Histologic evaluation of the tumor suggested histologic grade III, and high expressions of estrogen and progesterone receptor (ER and PR, 95% and 80%, respectively) and negative human epidermal growth factor receptor 2 (HER2) on immunohistochemistry (IHC) testing. The patient received neoadjuvant chemotherapy (four cycles of adriamycin and cyclophosphamide followed by 4 cycles of docetaxel) and underwent total mastectomy with sentinel lymph node biopsy in January, 2014 which showed ypT1N0 tumor with ER/PR/HER2 95%/3%/`+ (negative) and Ki67 level of 5%. The patient received adjuvant endocrine therapy with tamoxifen. After no evidence of disease for 26 months, the patient developed recurrent lymph node metastasis in the ipsilateral axilla and underwent axillary lymph node dissection in March, 2016. Immunohistochemical evaluation of the lymph nodes showed ER/PR/HER2 80%/5%/3+ and Ki67 level of 15%. While the patient was receiving docetaxel+carboplatin+trastuzumab (TCH) for the HER2-positive disease, liver metastasis was detected after two cycles in June, 2016. Core needle biopsy of the liver suggested metastatic carcinoma with ER/PR/HER2 95%/1%/1+ and Ki67 3% on IHC. The patient was on disease progression during successive endocrine therapies with letrozole, exemestane, everolimus, and fulvestrant suggesting endocrine resistance when the blood sample was obtained.

The two pancreatic cancer patients analysed were diagnosed through AJCC 8th edition TNM staging system, from which pancreatic cancer patient 1 (male, age of 76) was diagnosed as T2N1M0 and pancreatic cancer patient 2 (female, age of 80) was diagnosed to have tumour in pancreas (>2 cm in diameter) and regional lymph node metastasis (T2N2M0).