1. Materials and methods

Glass slides (75×50×1 mm) were purchased from Corning Inc. (Corning, NY). SPR220-7 was purchased from MicroChem Corp. (Newton, MA). MF-CD26 was obtained from Rohm and Haas Electronic Materials (Marlborough, MA). Tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane was obtained from Pfaltz and Bauer, Inc. (Waterbury, CT). FC-70 (a mixture of perfluoro-tri-*n*-butylamine and perfluoro-di-*n*-butylmethylamine) was purchased from Hampton Research (Aliso Viejo, CA). Fetal bovine serum (FBS) was obtained from Invitrogen (Grand Island, NY). PBS buffer (0.1 M, pH7.4) was obtained from Lonza, Inc. (Allendale, NJ). H_2PtCl_4, sodium citrate, catalase, (3-glycidoxypropyl) trimethoxysilane (3-GPS), (3-Aminopropyl)triethoxysilane (APTES), hydrogen peroxide solution (35% wt in H_2O), NH_4F, HF, and HNO_3 were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). $CuSO_4$, $NH_2OH.HCl$, EDTA, NaN_3, ascorbate and 3-amino-1,2,4-triazole, red ink was purchased from Fisher Scientific and was diluted 10× before use. Polyvinyl chloride sealing tape was obtained from 3M (St. Paul, MN). Amorphous diamond-coated drill bits (0.031 inch cutter diameter) were purchased from Harvey Tool (Rowley, MA). All devices were designed as computer graphics using AutoCAD software and then printed out as transparency photomasks by CAD/Art Services, Inc. (Bandon, OR) with resolution at 10 μm. CYFRA21-21 and all antibodies were obtained from Abcam (Cambridge, MA). Bioactive EGF was purchased from Shenandoah Biotechnology, Inc (Warwick, PA). The SKBR-3, MCF-7 and MDA-MB-231 cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA). MCF-7 and MDA-MB-231 cells were grown in DMEM supplemented with 10% FBS. SKBR-3 cells were grown in McCoys 5A medium supplemented with 10% FBS. All of these breast cancer cell lines were incubated at 37 °C in a 5% CO_2 atmosphere.

2. Experiments

A. Peroxidase activity of PtNPs

To test the peroxidase activity, PtNPs was introduced to catalyze the oxidation of the colorimetric peroxidase substrates 3,3,5,5-tetramethylbenzidine (TMB) or di-azo-aminobenzene (DAB) (blue or brown solution, respectively) in the presence of H_2O_2. Similar to the peroxidase-catalyzed reaction, the reaction with TMB can be quenched by adding 3 M H_2SO_4, resulting in the blue-to-yellow transition. Figure S2b shows photographs and absorbance spectra of the quenched solution verses concentrations of PtNPs. It is apparent that the reaction rate is increased with increasing PtNP concentration.

B. V-Chip preparation

Methods for V-Chip production can be found from our previous publications.[1] Access holes were prepared using a diamond drill of 0.031-inch diameter. Each glass plate was treated by salinizing with tridecafluoro-1,1,2,2-tetrahydrooctyl-1- trichlorosilane to given a hydrophobic coating. To assemble V-Chip, fluorinert liquid FC-70 was served as a lubricant preventing air pockets during operation.

C. Preparation and conjugation of PtNPs.

PtNPs with an average diameter of 30 nm were prepared by modifying the previously reported methods.[2] The size distribution of PtNPs was characterized by using scanning electron microscope (SEM) and dynamic light scattering (DLS). To prepared antibody conjugated PtNPs, 10 µL 1 mg mL^{-1} monoclonal antibody was mixed with 500 µL 1 mg mL^{-1} PtNPs in PBS buffer with pH 6.5, which was kept at 4 ^{o}C for overnight. Next, BSA was added to block the surface of PtNPs with the final concentration of 1 % and the unconjugated antibody was removed by

dialysis. Finally, the antibody conjugated PtNPs were suspended in 500 µL 10 mM PBS solution (pH 7.2, 0.1% Triton X-100, 5% sucrose, 1% BSA).[3]

D. Capture antibody immobilization in ELISA wells.

The wells were cleaned by applying drops of piranha solution for 1 h, then rinsed with Millipore water and dried with nitrogen gas. After that, 10% (3-glycidoxypropyl) trimethoxysilane in toluene was added into each well using a pipette. The solution was kept in each well for 30 min, and then rinsed with fresh toluene to remove extra 3-GPS molecules. Then the glass slides were dried using dry nitrogen gas and baked at 120 ^{o}C for 30 min. Epoxy groups were covalently modified onto the surface in each well.

Capture antibodies were covalently immobilized in the ELISA wells by reaction with the epoxy group on the glass surface. The capture antibodies were carefully added into each well and incubated at 4 ^{o}C overnight. Then the wells were washed with 1 % BSA several times to avoid nonspecific binding. The wet etched glass has a rough surface, which increases the coating efficiency of antibodies.

E. V-Chip ELISA reaction detection of CYFRA21-1.

For the detection of CYFRA21-1, fifty microliter different concentrations of CYFRA21-1 solution (0, 0.5, 1, 3, 10, 25 and 50 ng mL^{-1} in PBS buffer, pH 7.4 or serum) was carefully added to the assay well in the top plate with a pipette and incubated at room temperature for 0.5 h. The well was then washed with washing solution (1% BSA and 0.05% Tween-20 in PBS buffer) three times. next, 50 µg mL^{-1} PtNPs-antibody was added in the ELISA well, incubated for another 0.5 h, and washed four times with washing solution and once with PBS buffer. Subsequently, the bottom slide, coated with FC-70, was placed onto the top glass slide. Finally,

the ELISA chamber was washed with PBS buffer and the readout was obtained by sliding the V-Chip to allow the PtNPs probe to make contact with hydrogen peroxide.

F. Detection of HER2 and phosphorylated HER2 (pHER2) on cell surface

The wells were first treated with APTES to modify with amino groups to increase cell adhesion. The wells were cleaned by applying drops of piranha solution for 1 h, then rinsed with Millipore water and dried with nitrogen gas. After that, two microliter 2 % APTES in acetone was carefully added to each well with a pipette and incubated at room temperature for 10 min. Then the wells rinsed with fresh acetone to remove extra APTES molecules and dried with nitrogen gas. Amino groups were covalently modified onto the surface in each well.

PDMS elastomer was punched with uniformed oval shape holes and bonded to the bottom slide by carefully aligned with amino groups modified wells. After sterilization, 30 μL cells were added in each well and cultured overnight at 37 °C in a 5 % CO_2 atmosphere. To stain cells using peroxidase-like activity, the wells seeded with cells were washed with PBS buffer, fixed with 4% formaldehyde and permeabilized with 0.1% Triton X-100. After blocking with 2 % BSA, 50 μg mL^{-1} PtNPs-antibodies were added in each well, incubated for 1 h and washed three times with PBS buffer. Freshly prepared DAB and $H_2O_2$was added for color development.

To detect of HER2, the wells seeded with cells were washed with PBS buffer, fixed with 4% formaldehyde and permeabilized with 0.1% Triton-X-100. To detect pHER2, the cells were first treated with or without 100 ng·mL^{-1} EGF and 5 mM CI-1033 for 1 h and then fixed with 4% formaldehyde, permeabilized with 0.1% Triton X-100. After blocking with 2 % BSA, 50 μg mL^{-1} PtNPs-antibodies were added in each well, incubated for 30 min and washed three times with PBS buffer. The PDMS mode was peeled off and a few cells around the wells were removed by scraping. Subsequently, the top slide, coated with FC-70, was placed onto the bottom glass slide.

Finally, the fluidic path seeded with cells was loaded with PBS buffer and the readout was obtained by sliding the V-Chip to allow the PtNPs probe to make contact with hydrogen peroxide.

Reference

[1] a) Y. Song, Y. Zhang, P. E. Bernard, J. M. Reuben, N. T. Ueno, R. B. Arlinghaus, Y. Zu, L. Qin, *Nat Commun* **2012**, *3*, 1283; b) Y. Song, Y. Wang, L. Qin, *J Am Chem Soc* **2013**, *135*, 16785-16788.

[2] M. Huang, Y. Jin, H. Jiang, X. Sun, H. Chen, B. Liu, E. Wang, S. Dong, *J Phys Chem B* **2005**, *109*, 15264-15271.

[3] H.-C. Lin, I. L. Wang, H.-P. Lin, T. C. Chang, Y.-C. Lin, *Sensors and Actuators B: Chemical* **2011**, *154*, 185-190.

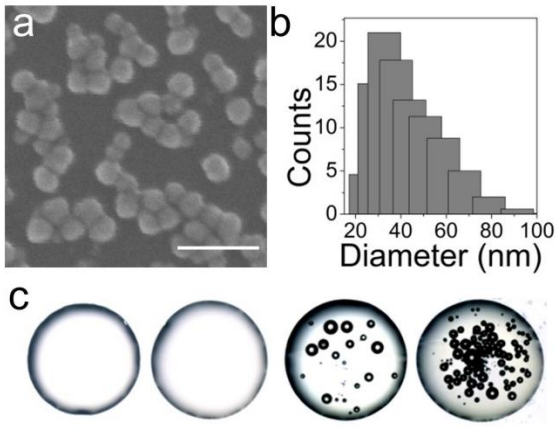

Figure S1. (a) SEM image of PtNPs. Scale bar, 100 nm. (b) Size distribution of PtNPs determined by dynamic light scattering (DLS). (c) The characteristic oxygen gas bubbles were observed when a drop of PtNPs was added. From left to right: 35 % H2O2, PtNPs solution, 35% H_2O_2 added with a drop of 10 or 30 μg·mL^{-1} PtNPs solution.

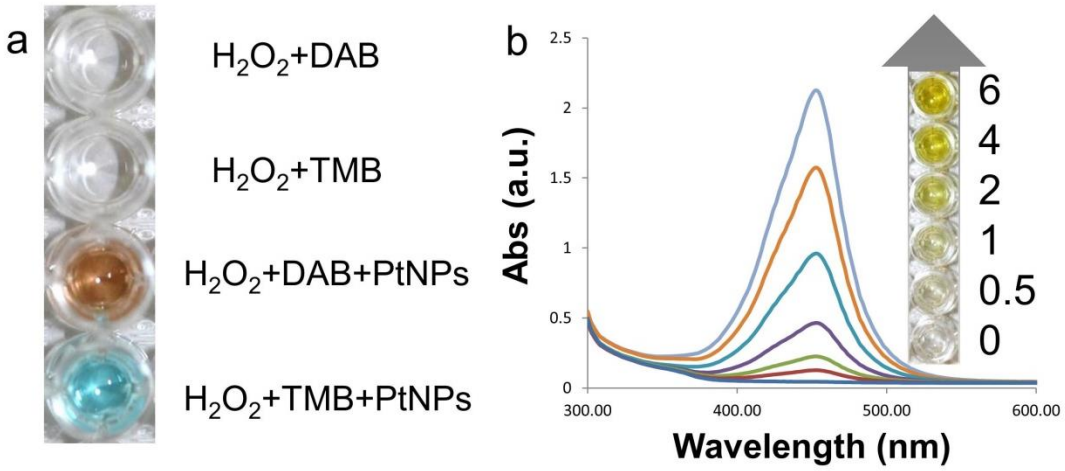

Figure S2. (a) PtNPs catalyze peroxidase substrates DAB and TMB in the presence of H_2O_2 to give a color product. (e) Photograph and absorbance spectra of TMB and H_2O_2 in absence (0 μg mL^{-1}) or presence of different concentration of PtNPs (0.5, 1, 2, 4 and 6 μg mL^{-1}).

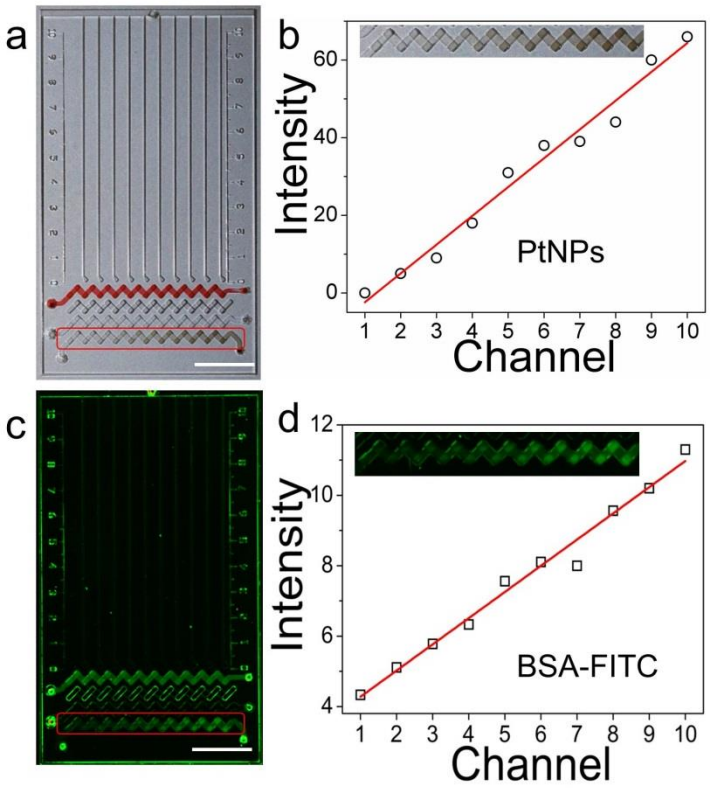

Figure S3. (a, c) A concentration gradient was generated by loading 2 μL PtNPs or BSA-FITC from the right bottom hole. (b, d) The intensity in arbitrary units of the diffused PtNPs or BSA-FITC at the center of each bottom wells. Scale bar is 1 cm for (a) and (c).The data was obtained by measuring the center of the bottom wells.

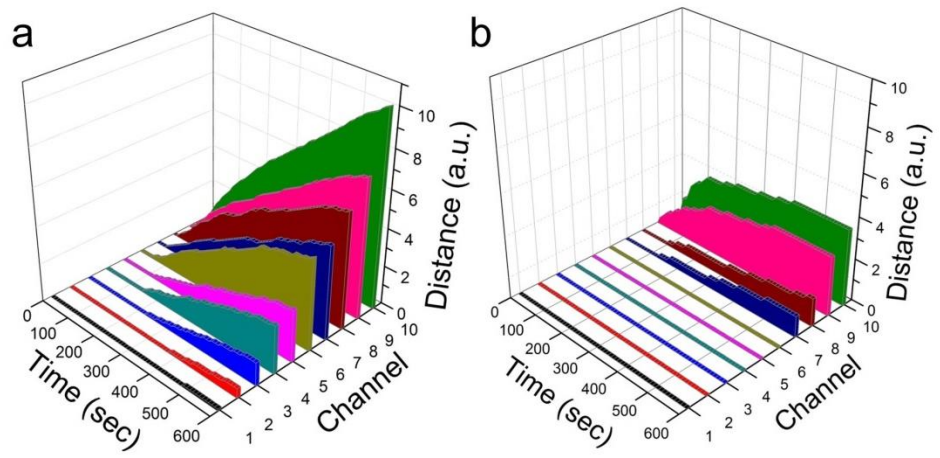

Figure S4. (a, b) The time dependent distance changes of each channel in 10 min diffusion of 2 μl PtNPs from the right loading holes.

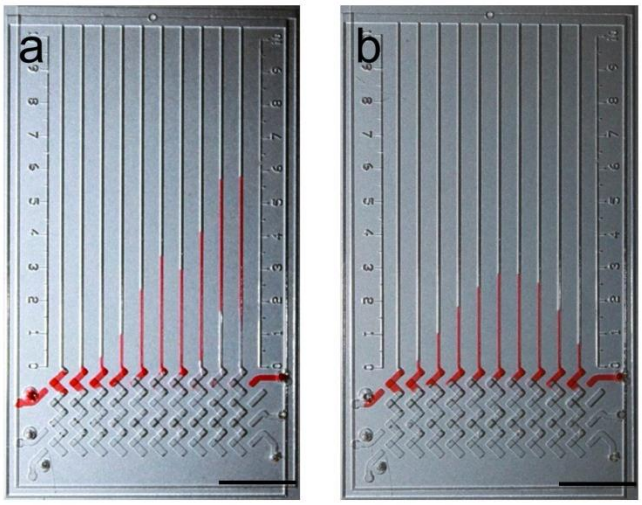

Figure S5. (a, b) Bar chart advancements of diffusing 2 µL 35 % H_2O_2 in the presence of 20 µg mL^{-1} PtNPs (a) or catalase (b). Scale bar, 1cm for (a) and (b).

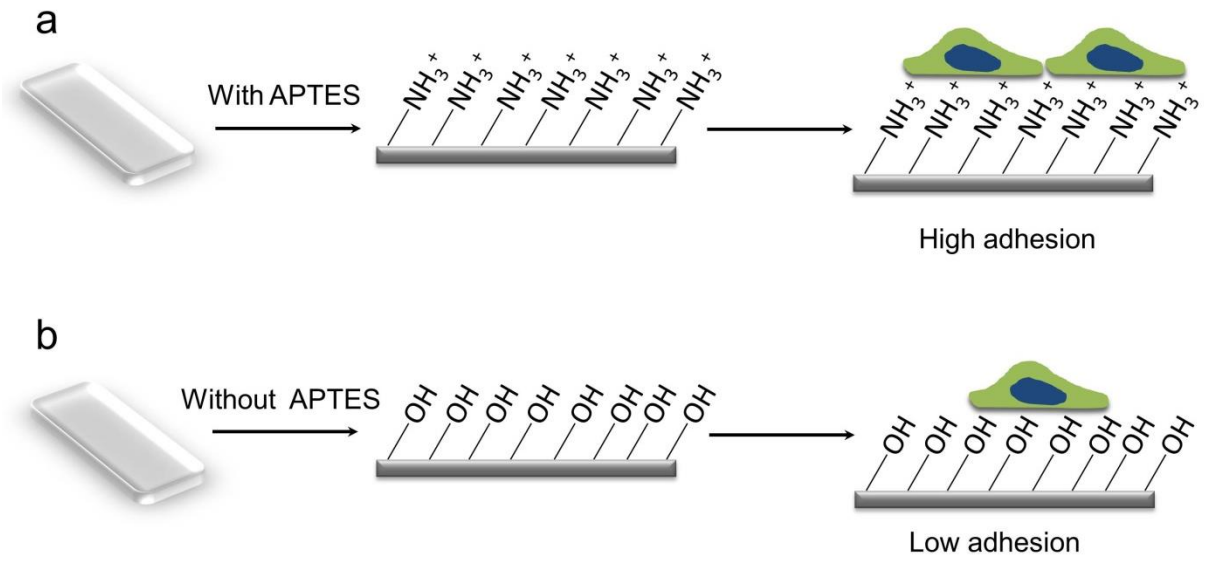

Figure S6. (a, b) Schematic images show the assay wells modified with amino-groups exhibit higher adhesion for cells than wells without modification.

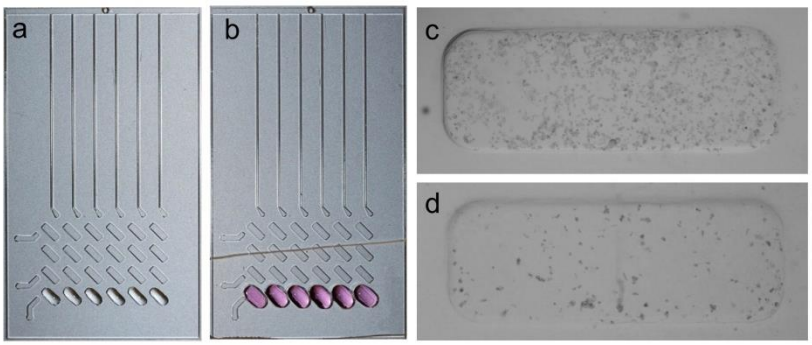

Figure S7. (a) 2 % APTES was loaded in each well for surface modification. (b) Cancer cell culture in the assay wells. Punched PDMS stamp was aligned with the wells for loading medium. (c, d) The assay wells modified with amino-groups exhibit higher adhesion (c) for cancer cells than wells without modification (d).

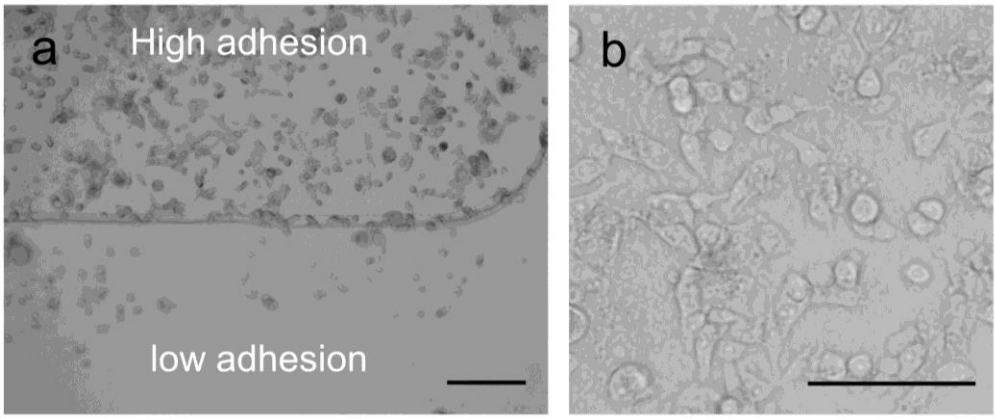

Figure S8. (a) The assay wells modified with amino-groups exhibit higher cell density in well than the area without modification. Scale bar, 200 μm. (b) MCF-7 cells exhibit spreading morphology in the APTES treated well. Scale bar, 100 μm.

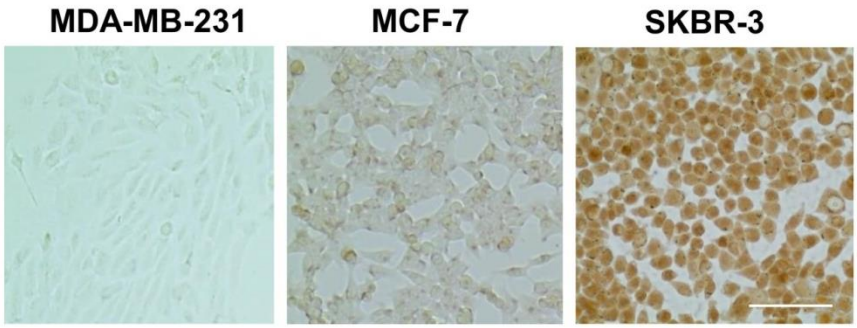

Figure S9. Immunostaining HER2 expression on MDA-MB-231, MCF-7 and SKBR-3 cells based on peroxidase-like activity of PtNPs. Scale bar, 1 cm.

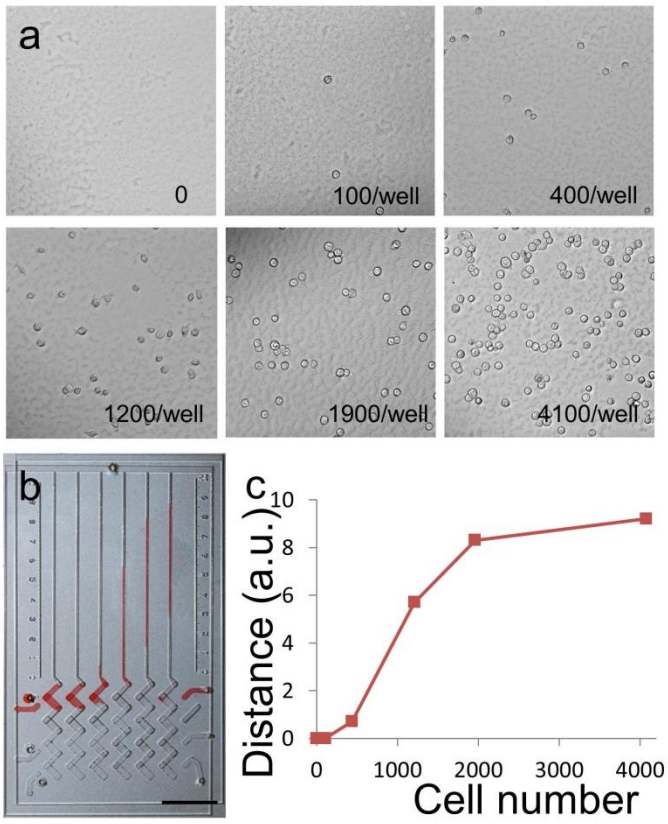

Figure S10. (a) SKBR3 cells distribution in each assay well. (b.c) Cell number dependent advancements by detection of HER2 expression on SKBR-3 cells. The advancements of the chip suggested PtV-Chip can detect as low as 400 SKBR3 cells. Scale bar, 1 cm for b.