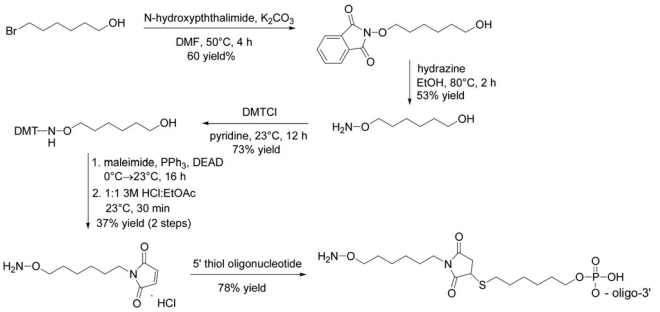
Supporting Information

Kazane et al. 10.1073/pnas.1120682109

SI Materials and Methods

Synthesis of Aminooxy-Modified Oligonucleotide. The aminooxy-maleimide linker was synthesized following a previously reported method (1). NMR and mass spectrometry were consistent with reported values (2). Electrospray ionization (ESI)-TOF high-accuracy: 213.1 [M+H]. The 5′ thiol oligonucleotide (IDT Technologies, 100 μM) was reduced with 5 mM *tris*(2-carboxyethyl) phosphine (TCEP) for 2 h at room temperature in PBS (pH 7.5), and 10 mM maleimide linker was added. After 1.5 h, the reaction was purified on an anion exchange column (Mono Q 5/50 GL, Buffer A: 20 mM Tris, 10 mM NaCl, pH 7.5; Buffer B: 20 mM Tris, 2 M NaCl, pH 7.5), desalted (PD10 column), and lyophilized. Yield, 78%.



1. Hutchins BM, et al. (2011) Selective formation of covalent protein heterodimers with an unnatural amino acid. *Chem Biol* 18:299–303.

2. Berndt M, Pietzsch J, Wuest F (2007) Labeling of low-density lipoproteins using the 18F-labeled thiol-reactive reagent N-[6-(4-[18F]fluorobenzylidene)aminooxyhexyl] maleimide. *Nucl Med Biol* 34:5–15.

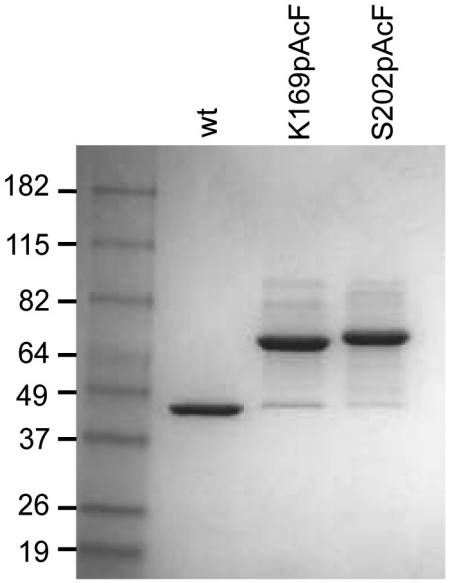


Fig. S1. Crude gel from anti-Her2 Fab-oligonucleotide reaction. The aminooxy-oligonucleotide (3 mM) was added to 100 μM anti-Her2 Fab with 100 mM methoxy aniline in acetate buffer pH 4.5. The reaction was incubated for 16 h at 37 °C and then resolved on an SDS-PAGE gel. The gel was stained with GelCode Blue (Pierce). Over 90% of the anti-Her2 Fab coupled to the oligonucleotide as seen by gel shift and densitometry.

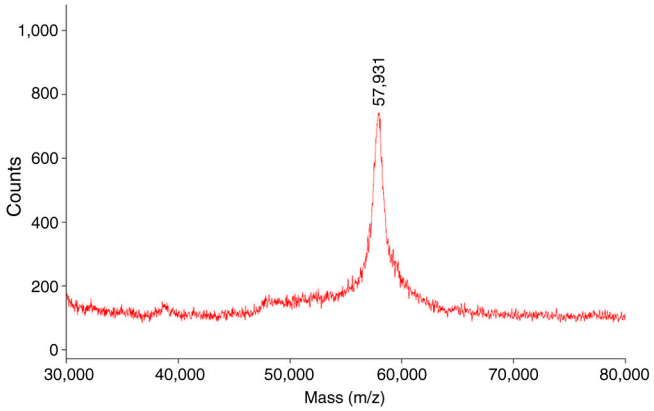


Fig. S2. MALDI-TOF of anti-Her2 Fab K169pAcF-oligonucleotide conjugate (where pAcF is *p*-acetylphenylalanine). Expected: 58,041. Actual: 57,931.

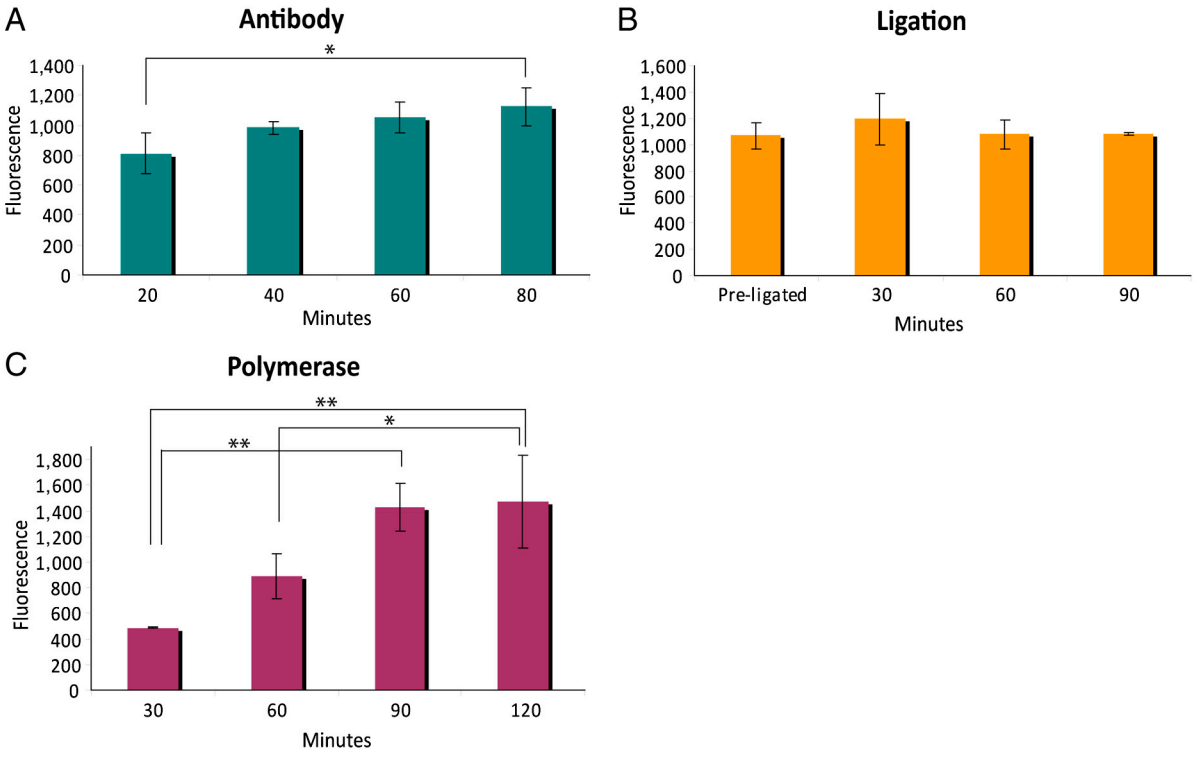


Fig. S3. Immuno-PCR time points. The antibody-oligonucleotide conjugate (S202pAcF oligobody, where pAcF is *p*-acetylphenylalanine), T4 ligase, and phi29 polymerase were added at different times in a 96-well tissue culture treated plate to determine optimal immuno-PCR conditions. (*A*) Antibody-oligonucleotide conjugate (2 μg/mL) was added for 20, 40, 60, or 80 min (with 60 min being the standard procedure). Ligase (0.05 U/μL) was added for 60 min, polymerase (0.125 U/μL) was added for 90 min, and Alexa Fluor 488 complementary oligonucleotide (62.5 nM) was added for 60 min. One-way ANOVA test indicated p value <0.05. (*B*) Ligase was either preligated with the antibody-oligonucleotide conjugate for 60 min at 37 °C before adding to cells or ligase was added for 30, 60, or 90 min. One-way ANOVA test indicated p value >0.05. (*C*) Polymerase was added for 30, 60, 90, or 120 min. One-way ANOVA test indicated p value <0.002. Fluorescence was quantified using ImageJ. Error bars represent the standard deviation between the average signal of triplicate frames. Tukey's post hoc test: p value *<0.05, **<0.01, ***<0.001.

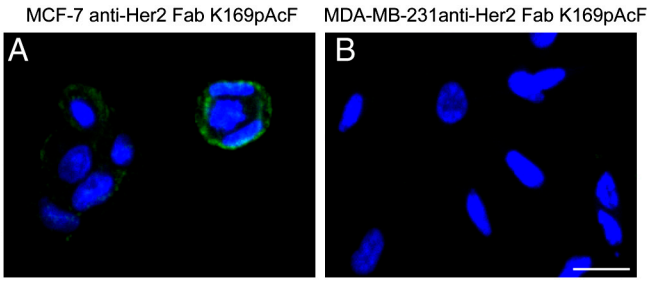


Fig. S4. Immuno-PCR on Her2^{Lo} cell line. Immuno-PCR with anti-Her2 Fab K169pAcF, where pAcF is *p*-acetylphenylalanine, (2 μg/mL) was performed as previously described. Alexa Fluor 488 complementary oligonucleotide (62.5 nM) was used for detection. No signal was observed for the Her2^{-} cell line (MDA-MB-231), whereas there was detectable signal for the Her2^{Lo} cell line (MCF-7). Exposure time for each cell line was 600 ms. Scale bar, 25 μm.

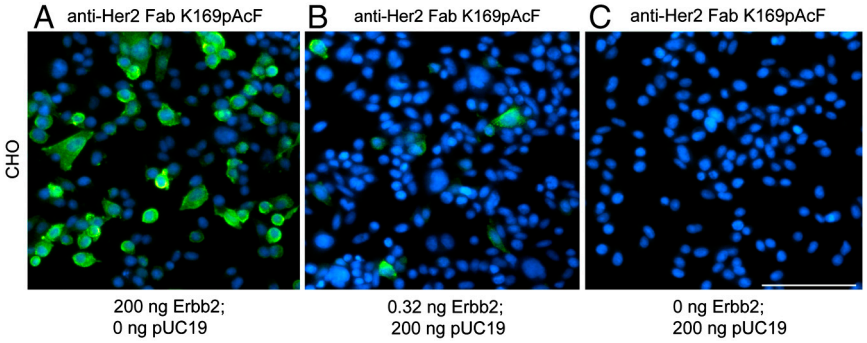


Fig. S5. Immuno-PCR on Her2 transfected CHO cells. CHO cells were grown in DMEM F12 with 10% FBS. At approximately 90% confluency, they were trypsinized and split 1:2.5 into a 96-well tissue culture treated plate. After 24 h, pUC19 and pCMV4 encoding Her2 were added at different concentrations to Opti-MEM I Reduced Serum Medium (Invitrogen). pUC19 was used to normalize the amount of DNA for each transfection. Lipofectamine 2000 (0.5 μL/well) (Invitrogen) was added to Opti-MEM separately, and after a 5 min incubation, the diluted DNA and Lipofectamine 2000 were mixed together. Fifty microliters was added to each well and incubated for 24 h at 37 °C. Immuno-PCR, as described previously, was performed using the anti-Her2 Fab K169pAcF-oligonucleotide conjugate, where pAcF is *p*-acetylphenylalanine, (2 μg/mL). When a high concentration of Her2 DNA was added, a large number of cells were transfected and a fluorescent signal (green) was detected (*A*). When a low amount of Her2 DNA was added, only a few cells are transfected with Her2 and fewer cells have signal (*B*). When no Her2 DNA was added, the cells were only transfected with pUC19 and no signal was observed (*C*). Nuclei were stained with Hoechst 33342 (blue). Scale bar, 100 μm.

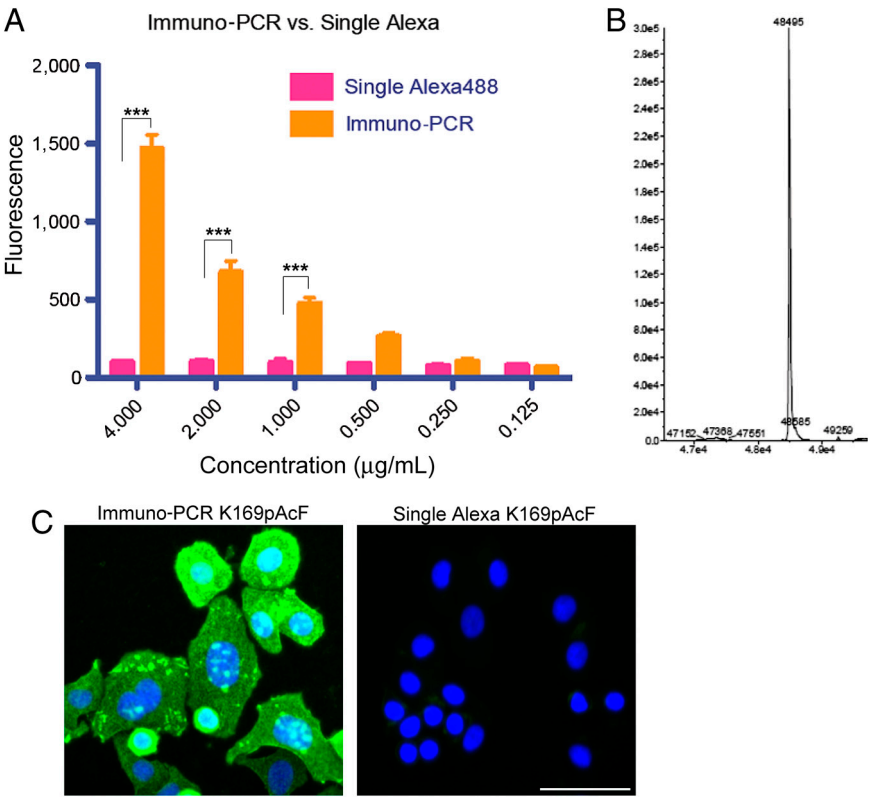


Fig. S6. (*A*) Antibody titration curve. Her2 K169pAcF Fab, where pAcF is *p*-acetylphenylalanine, was site-specifically conjugated to aminooxy-Alexa Fluor 488 as described previously (1). Different concentrations of Her2 K169pAcF-Alexa Fluor 488 or Her2 K169pAcF-oligonucleotide were added to cells. Immuno-PCR was performed on the antibody-oligonucleotide conjugate. Fluorescence was quantified using ImageJ. Error bars represent the standard deviation between the average signal of triplicate frames. One-way ANOVA test indicated *p* value <0.001. Tukey's post hoc test: *p* value *<0.05, **<0.01, ***<0.001. (*B*) ESI-MS of Her2 K169X-Alexa Fluor 488. Expected: 48,493. ESI-MS: 48,495. (*C*) Fluorescent microscope image of immuno-PCR with Her2 K169pAcF-oligonucleotide or staining with Her2 K169pAcF-Alexa Fluor 488 at 4 μg/mL concentration. Scale bar, 50 μm.

1. Hutchins BM, et al. (2011) Site-specific coupling and sterically controlled formation of multimeric antibody fab fragments with unnatural amino acids. *J Mol Biol* 406:595–603.

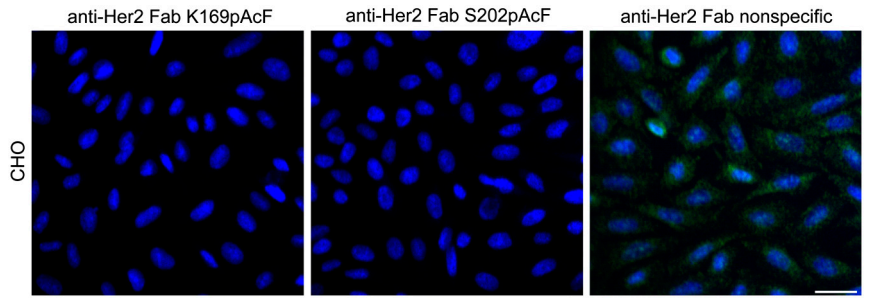


Fig. S7. Comparison of site-specific and randomly labeled Her2 on CHO cells (Her2^{-}). Immuno-PCR was performed as previously described with Her2 *p*-acetylphenylalanine (pAcF) oligobodies (K169pAcF or S202pAcF) and nonspecifically labeled Her2 Fab (2 μg/mL for each Fab). Alexa Fluor 488 complementary oligonucleotide (62.5 nM) was used for detection. No signal was observed for either of the site-specific conjugates (K169pAcF and S202pAcF), whereas the randomly labeled Her2 bound nonspecifically. Exposure time for each cell line was 200 ms. Scale bar, 25 μm.

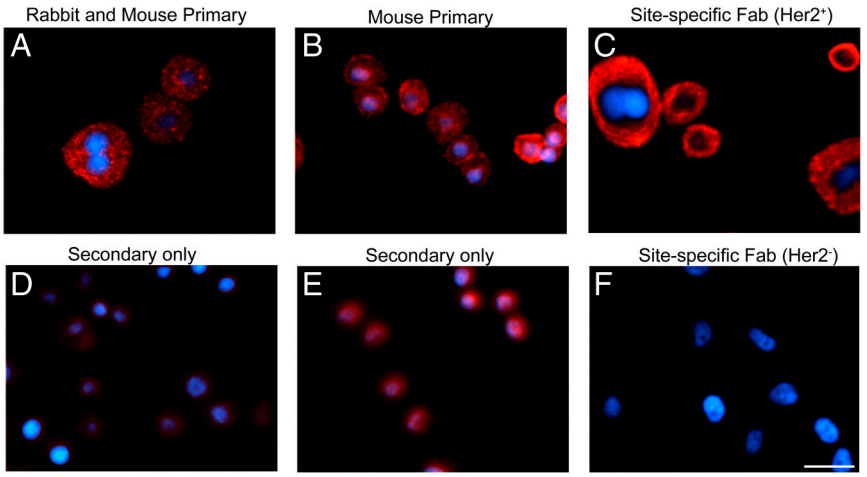


Fig. S8. Comparison with secondary antibody immuno-PCR. (*A*) Rabbit polyclonal antibody (Dako A0485) and mouse monoclonal antibody (Millipore Q5-1130, clone N3/D10) were used to detect SK-BR-3 cells. The rabbit and mouse antibodies bind to different epitopes of Her2. Secondary antibodies (anti-mouse PLUS and anti-rabbit MINUS) were purchased from Olink Bioscience (no. 90701 and no. 90602) for proximity ligation immuno-PCR. The procedure for immuno-PCR was taken from Olink Bioscience (Olink kit no. 90134). (*B*) Mouse monoclonal antibody (Millipore Q5-1130, clone N3/D10) was added to SK-BR-3 cells. Secondary antibodies (anti-mouse PLUS no. 90701 and anti-mouse MINUS no. 90601) were added for proximity ligation immuno-PCR to detect Her2. (*D–E*) To measure nonspecific binding, only the secondary antibodies were used per Olink's kit instructions. We compared detection with two primary antibodies and one primary antibody to the site-specific immuno-PCR using K169pAcF oligobody, where pAcF is *p*-acetylphenylalanine, (2 μg/mL) (as described previously) (*C* and *F*). Both procedures using secondary antibodies show nonspecific binding to cells (as demonstrated with the emission of primary antibodies), whereas the site-specific method does not show any detectable background. Cy3 was used as the detection fluorophore. Scale bar, 25 μm.

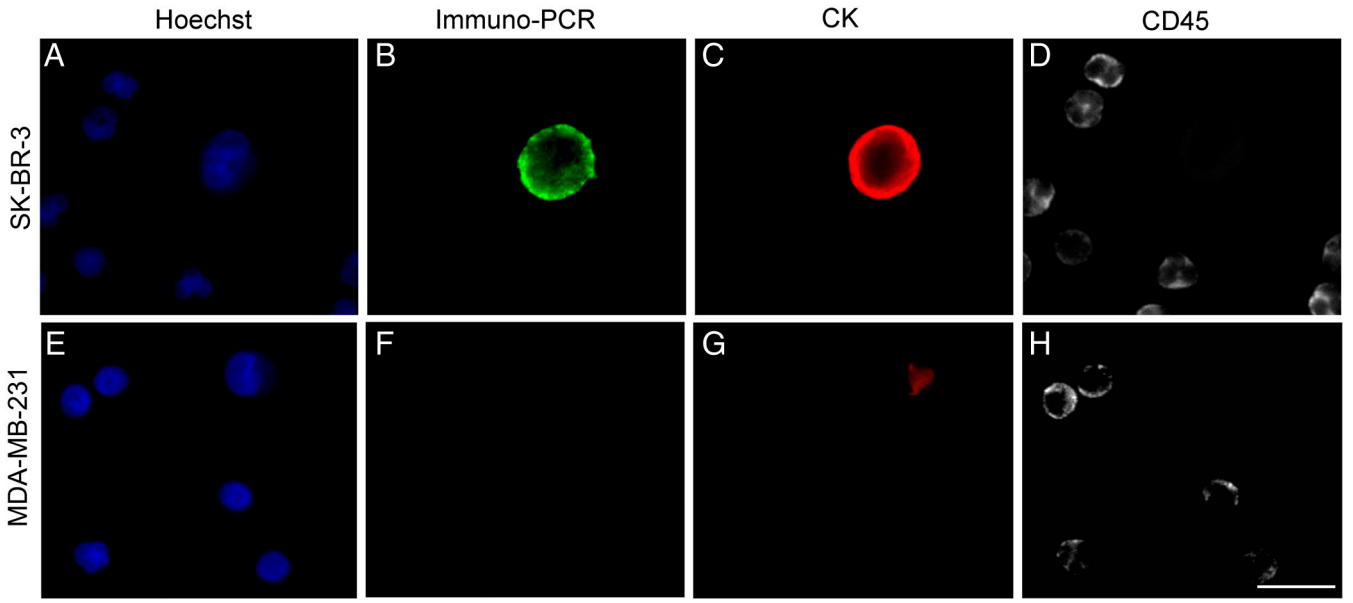


Fig. S9. Immuno-PCR on spiked cancer cells in whole blood. Red blood cells from normal donor blood were lysed and SK-BR-3 or MDA-MB-231 cells were spiked into WBCs (1:3,000). Approximately three million cells were plated on glass slides and immuno-PCR was performed with K169pAcF oligobody, where pAcF is *p*-acetylphenylalanine, (2 μg/mL) and the addition of anti-CK19 (1:100, Dako), anti-panCK (1:100, Sigma) and Alexa Fluor 647 anti-CD45 (1:125 AbD Serotec) primary antibodies. Cytokeratin (CK) primary antibodies were detected using an Alexa Fluor 555 secondary antibody (1:500). Slides were then sealed with cover glass and imaged using a customized high-throughput fluorescence microscope with a 10× magnification. The images were compiled in a four-channel assay. Cells were identified using Hoechst 33342 nuclear stain (blue) (*A*, *E*). The anti-CK antibodies (red) stained both the SK-BR-3 cells (*C*) and MDA-MB-231 cells (*G*), whereas the Her2 immuno-PCR (green) only stained SK-BR-3 cells (*B*, *F*). CD45 (gray) only stained WBCs (*D*, *H*). Staining of the anti-CK antibody is diminished in MDA-MB-231 cells because there was a lower expression of cytokeratin in these cells (1). Scale bar, 25 μm.

1. Sommers CL, et al. (1989) Vimentin rather than keratin expression in some hormone-independent breast cancer cell lines and in oncogene-transformed mammary epithelial cells. *Cancer Res* 49:4258–4263.

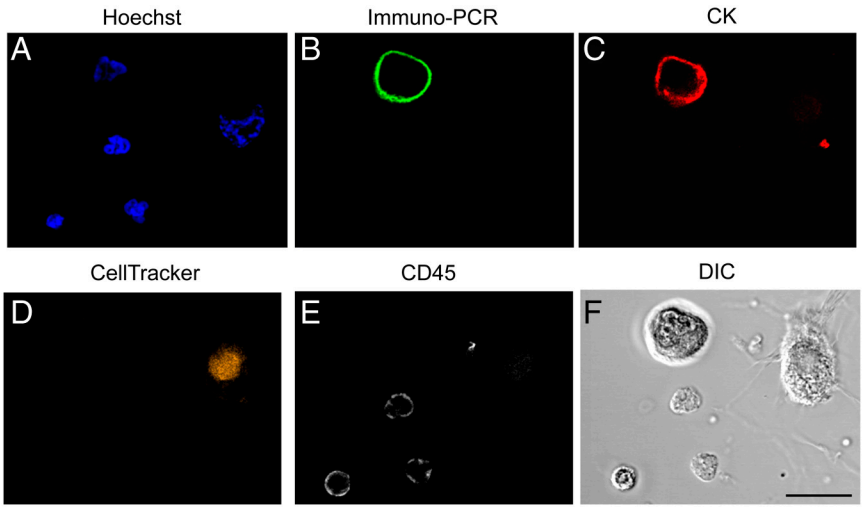


Fig. S10. Immuno-PCR on spiked cancer cells (both Her2 positive and negative) in whole blood. Red blood cells from normal donor blood were lysed and SK-BR-3 and MDA-MB-231 cells were spiked into WBCs (1:10). MDA-MB-231 cells were previously stained with CellTracker Red (Invitrogen) so they could be identified. Cells were plated on glass slides and immuno-PCR was performed with K169pAcF oligobody, where pAcF is *p*-acetylphenylalanine, (2 μg/mL) and the addition of anti-CK19 (1:100, Dako), anti-panCK (1:100, Sigma) and Alexa Fluor 647 anti-CD45 (1:125 AbD Serotec) primary antibodies. Cytokeratin (CK) primary antibodies were detected using an Alexa Fluor 555 secondary antibody (1:500). Slides were sealed and confocal images were taken. Individual channels are shown. Cells were identified using Hoescht (*A*). The cell tracker only stained MDA-MB-231 cells (*D*). Anti-CK antibodies stained both MDA-MB-231 cells and SK-BR-3 cells (*C*). Immuno-PCR only showed signal in SK-BR-3 cells (*B*). CD45 stained WBCs (*E*). Differential interference contrast (DIC) is also shown (*F*). Scale bar, 25 μm.