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Supporting Information

Detection of HER2$^+$ Breast Cancer Cells using Bioinspired DNA-Based Signal Amplification

Sarah D. Rafiee[+], Samet Kocabey[+], Michael Mayer, Jonathan List,* and Curzio Rüegg*

Author Contributions

S.R. Data curation:Equal; Formal analysis:Equal; Investigation:Equal; Methodology:Equal; Validation:Equal; Writing - Original Draft:Equal
S.K. Data curation:Equal; Formal analysis:Equal; Investigation:Equal; Methodology:Equal; Validation:Equal; Writing - Original Draft:Equal
M.M. Conceptualization:Supporting; Formal analysis:Supporting; Funding acquisition:Supporting; Methodology:Supporting; Resources:Supporting; Supervision:Supporting; Writing - Review & Editing:Equal
J.L. Conceptualization:Lead; Data curation:Equal; Formal analysis:Equal; Investigation:Supporting; Methodology:-Supporting; Supervision:Supporting; Writing - Original Draft:Equal
C.R. Conceptualization:Equal; Data curation:Equal; Formal analysis:Equal; Funding acquisition:Lead; Project administration:Lead; Resources:Lead; Supervision:Equal; Validation:Equal; Writing - Original Draft:Equal

Experimental Procedures

Materials

Native and biotin-modified DNA strands (HPLC purified) used for the screening on streptavidin coated beads (Cy5 labeled and HPLC purified) were purchased from Biomers (Ulm, Germany). Alexa-647 modified H1 and H2 DNA strands (HPLC purified) used in cell experiments were purchased from Eurofins MWG Operon (Ebersberg, Germany). The detailed sequences of the DNA strands are given in Table S1. Anti-human HER2/ERBB2 antibody (trastuzumab) was purchased from R&D Systems (United Kingdom). Streptavidin–Alexa Fluor 488 conjugate was purchased from Thermo Fisher Scientific (Basel, Switzerland).

Screening the hairpins with streptavidin beads by flow cytometry

2.5 µL of 0.5% w/v beads corresponding to 2.5 × 10^5 streptavidin coated polystyrene beads (4-4.9 µm diameter, Spherotech, Illinois, USA) were transferred to U-bottom 96-well microplate (Thermo Fisher Scientific (Basel, Switzerland)). Beads were incubated sequentially with double initiator (DI) (5 nM) for 20 minutes, H1-AF647 (500 nM) and H2 (500 nM) for different time intervals (30 minutes, 2 hours, 4 hours and 24 hours) in 100 µL of FACS buffer (5 mM ethylenediaminetetraacetic acid (EDTA), 3% FCS in 1x PBS) at 4 °C. Between each step, cells were rinsed with 200 µL FACS buffer and centrifuged at 260g (Awel MF48-R) for 2 minutes to wash unbound molecules away. Before adding DI, H1 and H2, oligonucleotides were incubated at 95 °C for 90 seconds and rapidly cooled on ice. After incubation for the respective times, the beads were analyzed using Flow Cytometry using MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec) and data analyzed with FlowJo Software (v10, FlowJo LLC). Median fluorescence intensity (MFI) for all samples were background corrected by subtracting the MFI values from the appropriate controls.

Cell culture

Human breast cancer cell lines BT-474 (high HER2/ERBB2 expression) was kindly provided by Dr. Mohamed Bentires-Alj (University of Basel, Switzerland). MDA-MB-468 (no detectable ERBB2 expression) and U937 (leukemia-derived monocytic cell line) were obtained from ATCC. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, 100 U/mL Penicillin and 100 µg/mL Streptomycin at 37 °C in 5% CO2. All cell culture reagents were purchased from Invitrogen Life Technologies (Basel, Switzerland).

HCR detection on cancer cells by flow cytometry

Cells cultured overnight were harvested with 1x trypsin (10x trypsin diluted in 1x PBS; Sigma-Aldrich (Buchs, Switzerland) and 2.5 × 10^5 cells were transferred to V-bottom 96-well microplate (Thermo Fisher Scientific (Basel, Switzerland)). Cells were washed with FACS buffer (5 mM ethylenediaminetetraacetic acid (EDTA), 3% FCS in 1x PBS) by centrifuging at 900 g, 4 °C for 5 minutes. Then, the supernatant was removed and cells were incubated sequentially with biotinylated trastuzumab (100ng/mL) for 30 minutes, streptavidin-AF488 (10µg/mL) for 20 minutes, double initiator (DI) (5 nM) for 20 minutes, H1-AF647 (500 nM) and H2 (500 nM) for different time intervals (30 minutes, 2 hours and 4 hours) in 100 µL of FACS buffer at 4 °C. Between each step, cells were rinsed with 200 µL FACS buffer and centrifuged at 700 g (Awel MF48-R) for 5 minutes to wash unbound molecules away. Before adding DI, H1 and H2, oligonucleotides were incubated at

95 °C for 90 seconds and rapidly cooled on ice. After incubation for the respective times, the cells were analyzed by flow cytometry using MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec) and data analyzed with FlowJo Software (v10, FlowJo LLC). Median fluorescence intensity (MFI) for all samples were background corrected by subtracting the MFI values from the appropriate controls.

HCR detection in co-cultured cancer cells by confocal microscopy

To visualize the selective targeting of $HER2^+$ overexpressing BT-474 cells, MDA-MB-468 cells ($HER2^-$) were stained with 1 µM Cell Tracker Green CMFDA (chloromethyl fluorescein diacetate, Invitrogen) for 30 minutes prior to mix with BT-474 cells according to the manufacturers protocol. Then, BT-474 cells and MDA-MB-468 cells were seeded into 24-well plates at a density of $2x10^4$ cells per well overnight. 13 mm glass coverslips were coated with 200 µL poly-L-lysine (Sigma Aldrich (Buchs, Switzerland)) for the adhesion of the cells. Next day cells were washed with 1x PBS and HCR was performed using biotinylated trastuzumab (100ng/ml), DI (5 nM), H1-Alexa647 (500 nM) and H2 (500 nM) for 2 hours. After washing with PBS, cells were fixed for 10 minutes using 4% formaldehyde solution (Sigma Aldrich (Buchs, Switzerland)). Fixed cells then washed with 1x PBS and mounted on glass slides using antifade mounting medium (Invitrogen). Confocal imaging was performed on a Leica TCS SP5 confocal microscope (Leica Microsystems GmbH, Mannheim, Germany).

HCR detection on cancer cells mixed with PBMC by flow cytometry

Peripheral blood mononuclear cells (PBMC) were isolated from the blood sample of a 25-years old healthy female donor. Ethical committee approval was obtained from the *Commission Cantonale de la recherche sur l'être humain of Canton Vaud*, Switzerland (023-CER-FR). Mononuclear cells were isolated from the blood using Histopaque-1077 (Sigma-Aldrich, (Buchs, Switzerland)) according to manufacturer's protocol. Briefly, 3 mL of whole blood was carefully layered on top of 3 mL Histopaque-1077 reagent in a 15 mL tube (9 mL blood was used in total) and centrifuged at 400 g (Awel C48) for 30 minutes at RT. After centrifugation, the opaque layer containing mononuclear cells were carefully aspirated and transferred to new tube. Then, the cells mixed with 10 mL 1x PBS and centrifuged again at 250 g (Awel C48) for 10 minutes. After several washing steps, the cell pellet was resuspended in 1x PBS and number of cells were counted. To investigate the sensitivity of the detection, BT-474 cells were mixed with PBMCs (10^5 cells/well) or U937 cells (10^5 cells/well) and seeded into V-bottom 96-well plates (Thermo Fisher Scientific (Basel, Switzerland)) at the following ratios: 1:1, 1:4, 1:10, 1:100. 2 hours after HCR, cells were washed and analyzed by Flow Cytometry as mentioned above.

HCR detection on cancer cells that directly spiked into the blood by flow cytometry

The blood sample was donated by a 31-years old healthy male donor. Ethical committee approval was obtained from the *Commission Cantonale de la recherche sur l'être humain of Canton Vaud*, Switzerland (023-CER-FR). BT-474 cells were counted and resuspended in 1x PBS and serially diluted. Then they mixed directly with 1 or 2 mL blood sample and further diluted at the following estimated ratios :1:10, 1:20, 1:30, 1:60, 1:100 and 1:200. Mononuclear cells mixed with BT-474 cells were isolated from the blood using Histopaque-1077 (Sigma-Aldrich, Buchs, Switzerland) according to manufacturer's protocol as briefly described above. All of the isolated cells were washed and analysed by Flow Cytometry after 2 hrs of HCR by choosing count limit as 3×10^6 cells per sample.

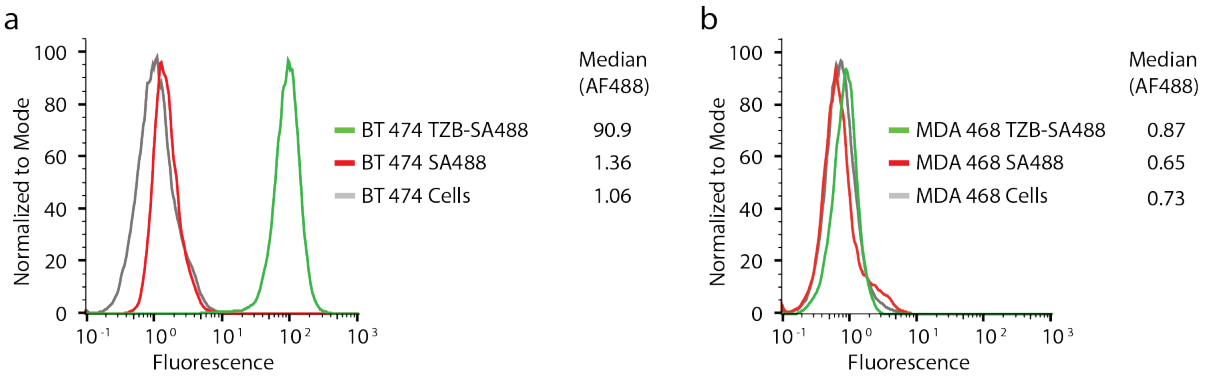

Figure S1. Association of biotinylated trastuzumab to a. HER2^{+} breast cancer cells BT-474 and b. HER2^{-} breast cancer cells MDA-MB-468.

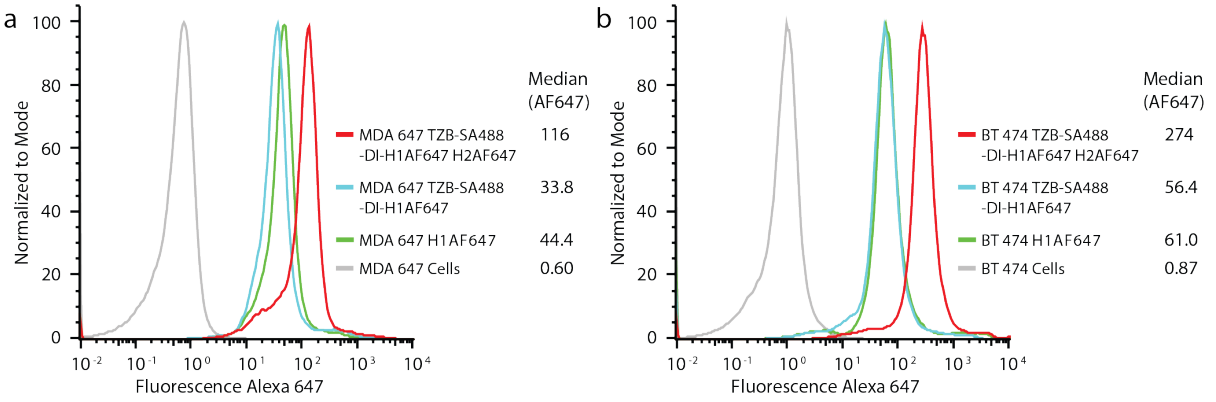

Figure S2. Hybridization Chain Reaction (HCR) at 37°C on (a) HER2^{-} breast cancer cells MDA-MB-468 and (b) HER2^{+} breast cancer cells BT-474.

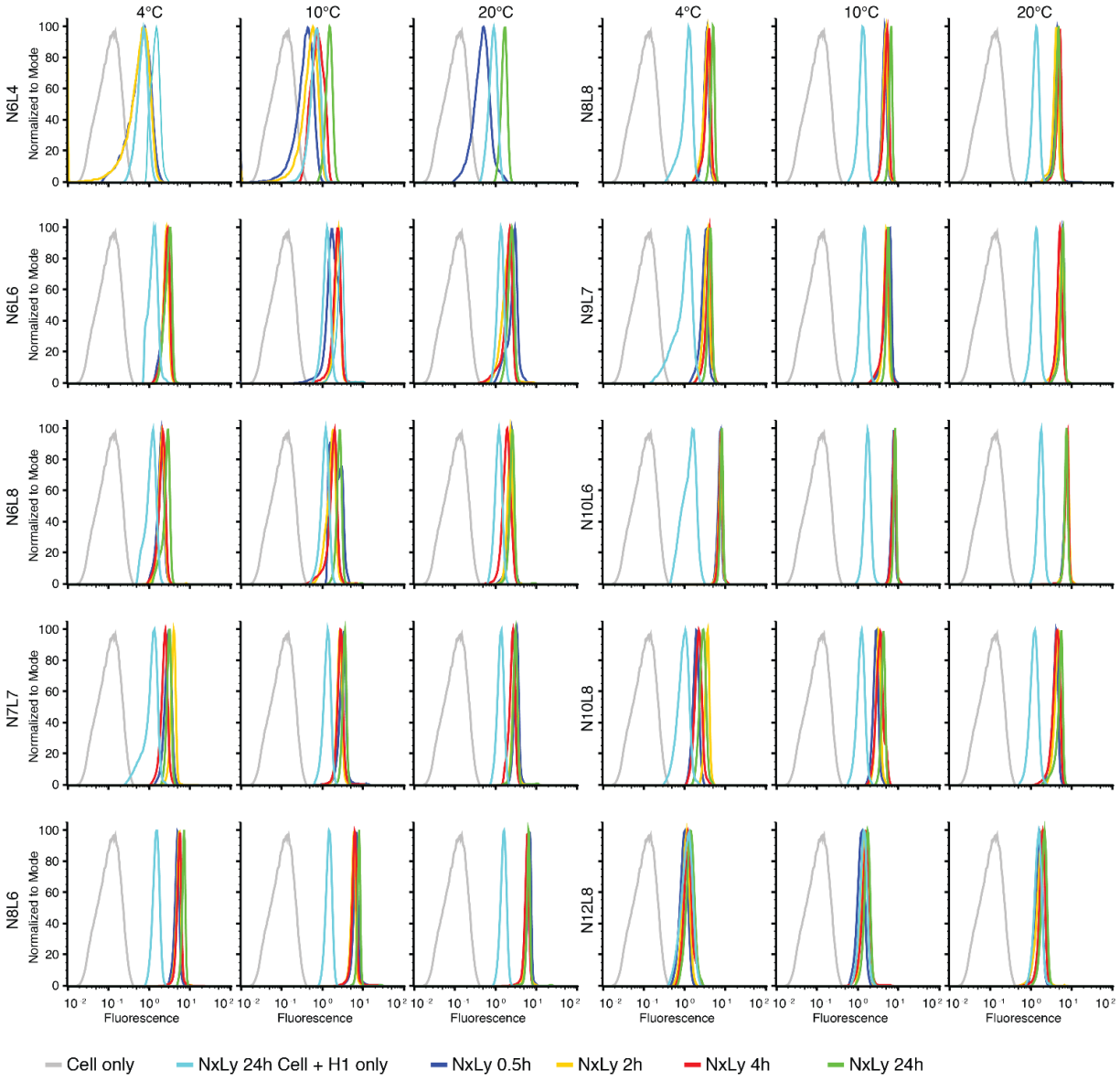

Figure S3. Screening of oligonucleotides using streptavidin-coated beads and flow cytometry analysis.

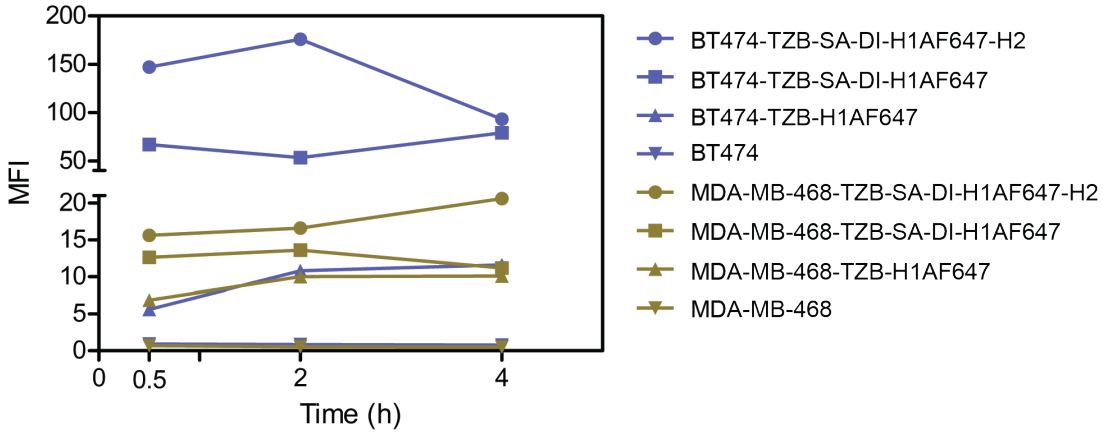

Figure S4. Time-dependent HCR amplification on BT-474 and MDA-MB-468 cells. (TZB: trastuzumab, DI: double initiator, H1&H2: hairpins, MFI: median fluorescence intensity)

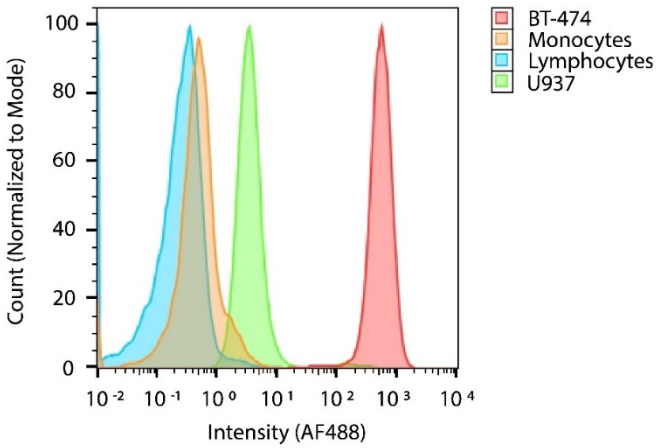

Figure S5. Binding of AF488-conjugated anti-HER2 antibody trastuzumab to the cells used in the detection experiments.

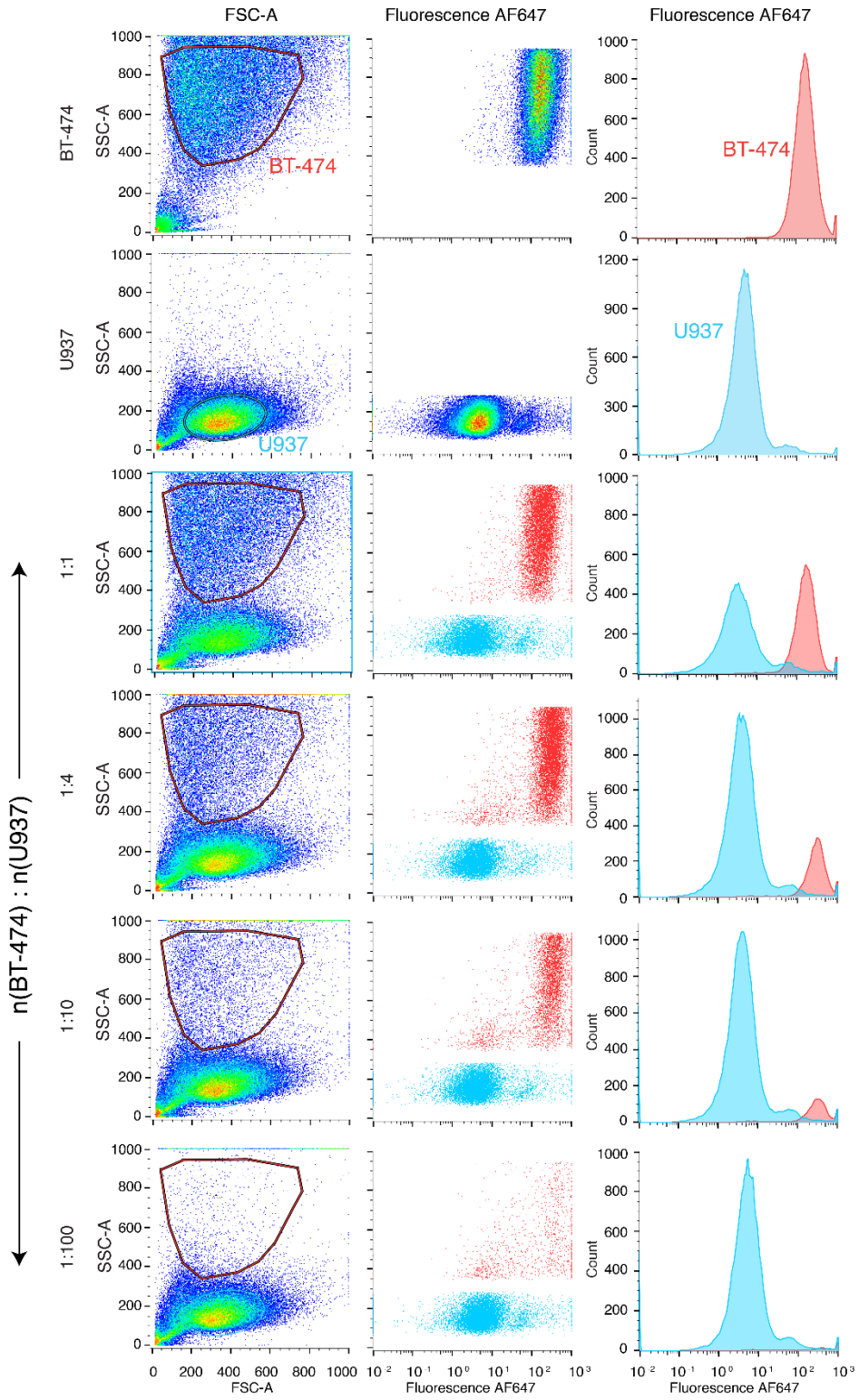

Figure S6. Detection sensitivity of HER2^{+} BT-474 cells co-cultured with U937 cells, by HCR amplification system.

Table S1. Double Initiator (DI) and hairpin sequences used in the present work. All sequences are listed 5' to 3'.

HCR N6L4	Sequence	5'-Mod.	Length
DI	TTCATAGTTCCGTTGTTCCGGTTC	Biotin	24
H1	TTGAACCGGAACCATAGTTCCG	Cyanine 5	22
H2	GTTCCGGTTCCGGAACTATG	None	20
HCR N6L6			
DI	TTCTTCTACCTCACTTCCTCACTATCAC	Biotin	28
H1	TTGTGATAGTGAGGCTTCTACCTCAC	Cyanine 5	26
H2	CCTCACTATCACGTGAGGTAGAAG	None	24
HCR N6L8			
DI	TTCTCTACTCCACCTCTTCACCTCACAATTAC	Biotin	32
H1	CACCTCACAATTACGAGGTGGAGTAGAG	Cyanine 5	28
H2	TTGTAATTGTGAGGTGCTCTACTCCACCTC	None	30
HCR N7L7			
DI	TTCTTCATTCATCTCCTTCATCTCCACTCTAC	Biotin	32
H1	CATCTCCACTCTACGGAGATGAATGAAG	Cyanine 5	28
H2	TTGTAGAGTGGAGATGCTTCATTCATCTCC	None	30
HCR N8L6			
DI	TTCACACTCAATCACCTTCAATCACCTCCAAC	Biotin	32
H1	TTGTTGGAGGTGATTGCACACTCAATCACC	Cyanine 5	30
H2	CAATCACCTCCAACGGTGATTGAGTGTG	None	28
HCR N8L8			
DI	TTCTTAACTCCATCTCACTTCATCTCACCATTACAC	Biotin	36
H1	TTGTGTAATGGTGAGATGCTTAACTCCATCTCAC	Cyanine 5	34
H2	CATCTCACCATTACACGTGAGATGGAGTTAAG	None	32
HCR N9L7			
DI	TTCCACTCACATCTCACCTTCATCTCACCTACATCC	Biotin	36
H1	TTGGATGTAGGTGAGATGCCACTCACATCTCACC	Cyanine 5	34
H2	CATCTCACCTACATCCGGTGAGATGTGAGTGG	None	32
HCR N10L6			
DI	TTCACCTCCACTTCACTCTTCACTTCACTCTTACTC	Biotin	36
H1	CACTTCACTCTTACTCGAGTGAAGTGGAGGTG	Cyanine 5	32
H2	TTGAGTAAGAGTGAAGTGCACCTCCACTTCACTC	None	34
HCR N10L8			
DI	TTCACCTCACCATCCAACTCTTCATCCAACTCAACTCCAC	Biotin	40
H1	TTGTGGAGTTGAGTTGGATGCACCTCACCATCCAACTC	Cyanine 5	38
H2	CATCCAACTCAACTCCACGAGTTGGATGGTGAGGTG	None	36
HCR N12L8			
DI	TTCTCATTCTCACTTACCTACCTTCACTTACCTACCATCCTCAC	Biotin	44
H1	TTGTGAGGATGGTAGGTAAGTGCTCATTCTCACTTACCTACC	Cyanine 5	42
H2	CACTTACCTACCATCCTCACGGTAGGTAAGTGAGAATGAG	None	40

Table S2. Corresponding number of gated cells and the median fluorescence intensities at AF488 and AF647 channels in Figure 4.

Sample Name	Subset Name	Count	Median (AF647)	Median (AF488)
BT-474	BT-474	36478	74.3	56.4
PBMCs	Monocytes	6445	6.57	1.93
	Lmyphocytes	29999	0.64	0.21
1 :1	BT-474	18150	74.3	50.3
	Monocytes	4063	4.15	1.9
	Lmyphocytes	17974	0.6	0.25
1 :4	BT-474	6533	99.5	64.2
	Monocytes	5517	4.22	1.78
	Lmyphocytes	25539	0.49	0.23
1 :10	BT-474	3396	108	72
	Monocytes	6132	4.24	1.74
	Lmyphocytes	28101	0.5	0.22
1 :1000	BT-474	861	106	70
	Monocytes	7375	4.66	1.75
	Lmyphocytes	33681	0.44	0.21

Table S3. Corresponding number of gated cells and the median fluorescence intensities at AF647 channel in Figure 5.

Sample Name	Subset Name	Count	Median (AF647)
1 :10	BT-474	146865	242
	Monocytes	182397	3.21
	Lmyphocytes	834000	0.25
1 :20	BT-474	101356	280
	Monocytes	241295	2.51
	Lmyphocytes	929000	0.14
1 :30	BT-474	49168	264
	Monocytes	193288	3.6
	Lmyphocytes	940000	0.31
1 :60	BT-474	34117	301
	Monocytes	197742	2.94
	Lmyphocytes	985000	0.24
1 :100	BT-474	23760	150
	Monocytes	146412	3.61
	Lmyphocytes	735000	0.33
1:200	BT-474	18301	490
	Monocytes	162794	2.99
	Lmyphocytes	852000	0.24