Supporting Information

Ultrasensitive Quantitation of Plasma Membrane Proteins via *is*RTA

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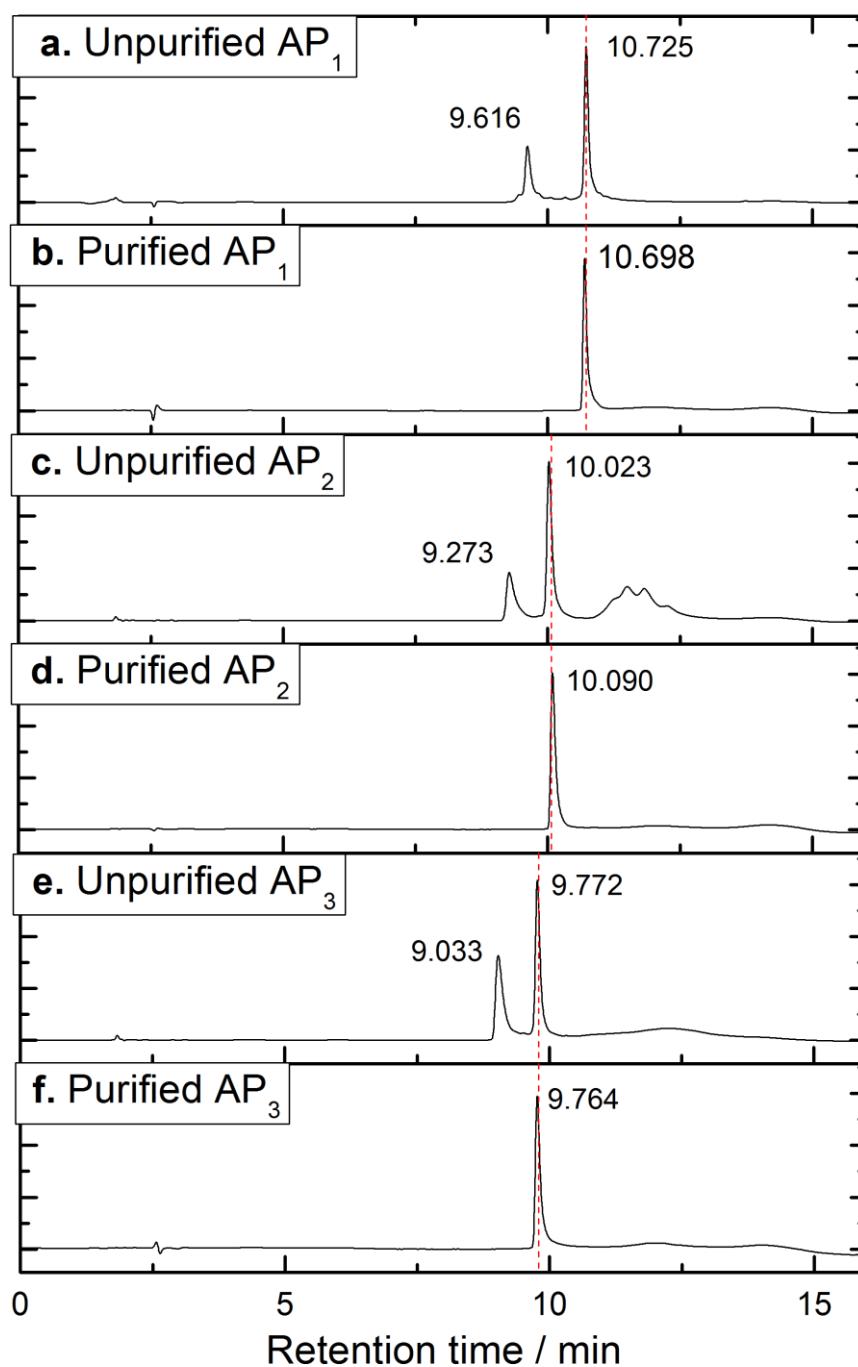
Figure S1. HPLC characterization and purification of the aptamer probes (**APs**). **a**, **c** and **e** showed the reaction mixtures of **AP₁**, **AP₂** and **AP₃**, respectively. **b**, **d** and **f** showed the corresponding **APs** after purification.
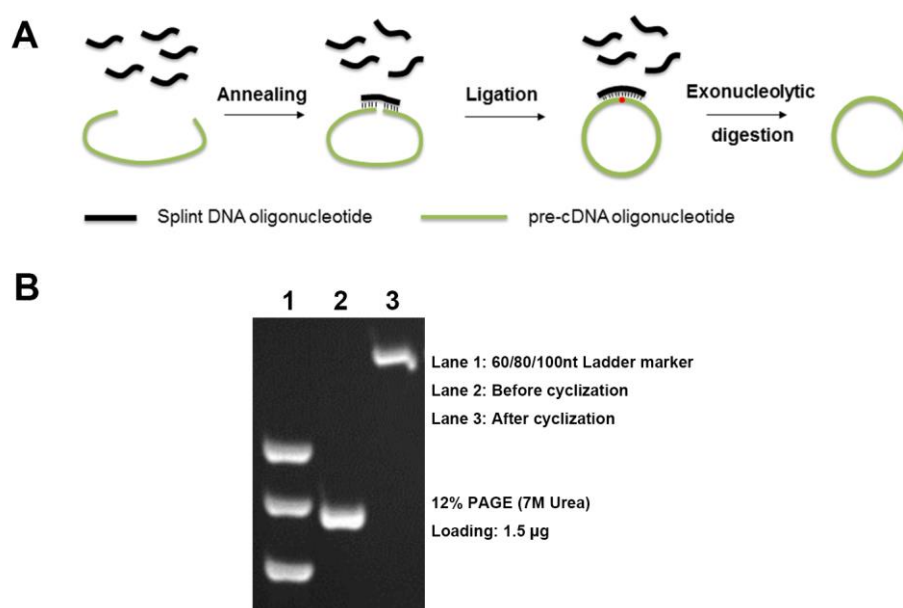
Figure S2. Generation of single-stranded circular template DNA (cDNA). (**A**) Schematic illustration shows the procedure for cDNA preparation. (**B**) PAGE assay of cDNA before (lane 2) and after (lane 3) cyclization reaction.
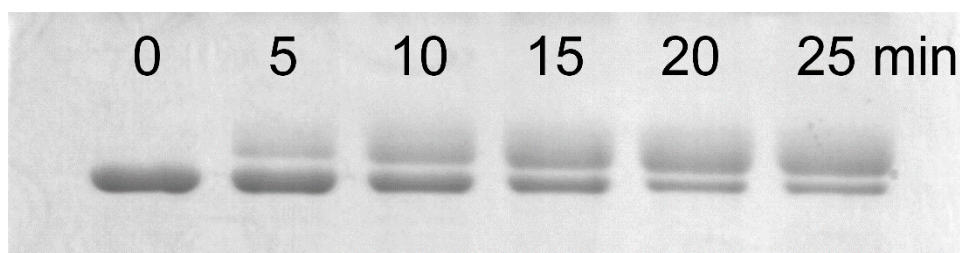
Figure S3. SDS-PAGE (12%) analysis of PMP labeling efficiency with **AP**s, displaying a time range of UV light (365 nm) irradiation with a loading sample containing 4.2 μM PMP (HER2 protein) and 10 μM **AP**. Increased band intensity of the AP-PMP conjugates (upper band) reveals a positive relationship between efficiency and irradiation time. And the irradiation time of at least 20 min is required to ensure an optimal conversion over 75%.
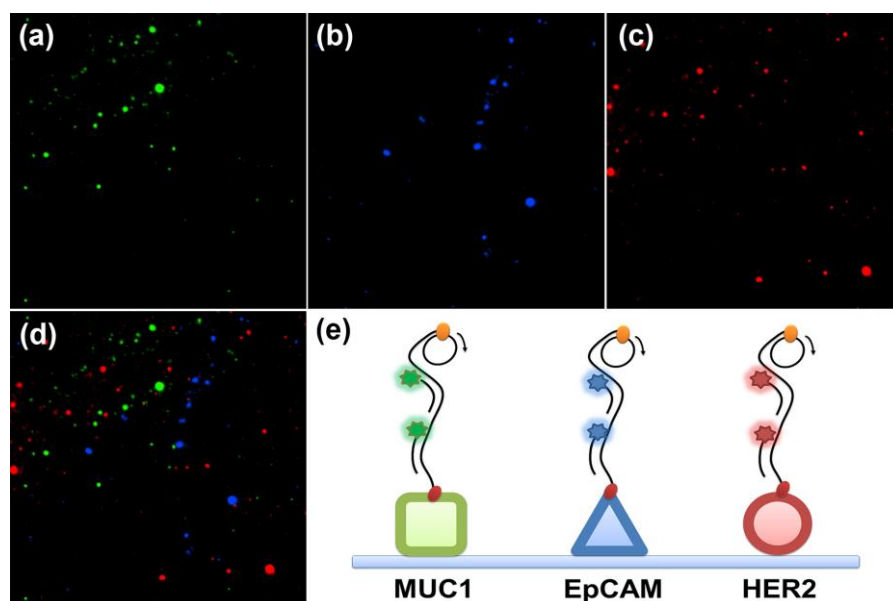
Figure S4. PMP labeling on glass slide surface. Fluorescence imaging of RCA amplicons (individual color spots) generated by corresponding AP-PMP conjunctions on the glass slide surface. Color-coded bright spots in these fluorescent images show locations of (a) **AP$_1$**-MUC1 (green), (b) **AP$_2$**-EpCAM (blue), (c) **AP$_3$**-HER2 (red) conjunctions, and (d) merged signals. Fluorescent signals were obtained by hybridization RCA amplicons with the corresponding iFBs that were modified with dyes of FAM (iFB$_1$), AMCA (iFB$_2$) and Cy3 (iFB$_3$).
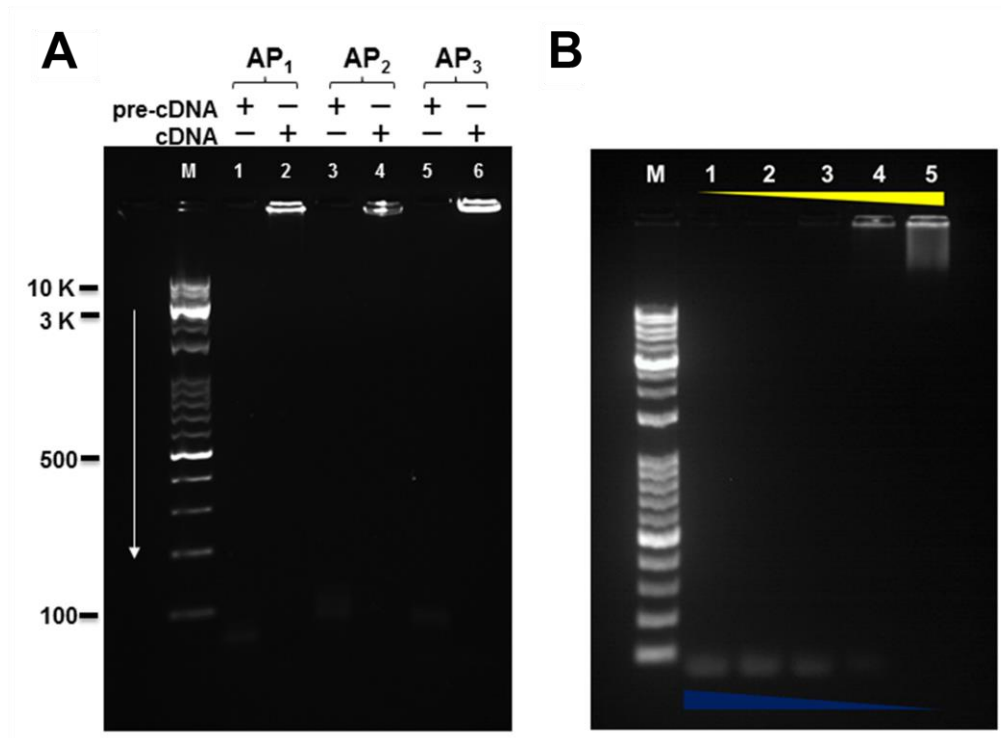
Figure S5. Feasibility of RCA reaction. (**A**) Agarose gel (1.5%) analysis of the RCA products by using cDNA and pre-cDNA pares. Lane M, 0.1-10 Kbp DNA ladder marker; Lanes 2, 4 and 6, RCA products by using 100 nM cDNA and corresponding **AP₁**, **AP₂** and **AP₃**; Lanes 1, 3 and 5, 100 nM each pre-cDNA were used for RCA. RCA products were not observed by using pre-cDNA, indicating RCA reaction couldn't be initiated by pre-cDNA. (**B**) Different **AP** concentrations (0, 1, 10, 100 and 1000 pM, lanes 1-5) were used to perform RCA reactions. Results show that the amount of RCA products increases with the increased of **AP** concentration.
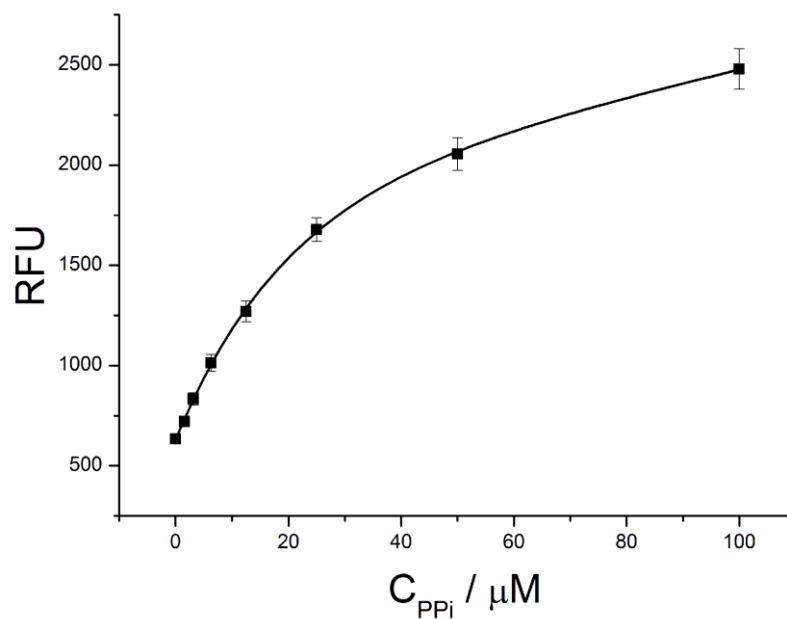
Figure S6. RCA products quantified by fluorimetric PPi assay. After RCA reaction, RCA products was removed with a QIAquick Nucleotide Removal Kit. The concentration of PPi in the reaction mixture was determined with a PhosphoWorks fluorimetric pyrophosphate (PPi) assay kit (AAT Bioquest, USA). The curve was stimulated by ExpAssoc model with an Origin 8.5 software, $r^2 = 0.999$.
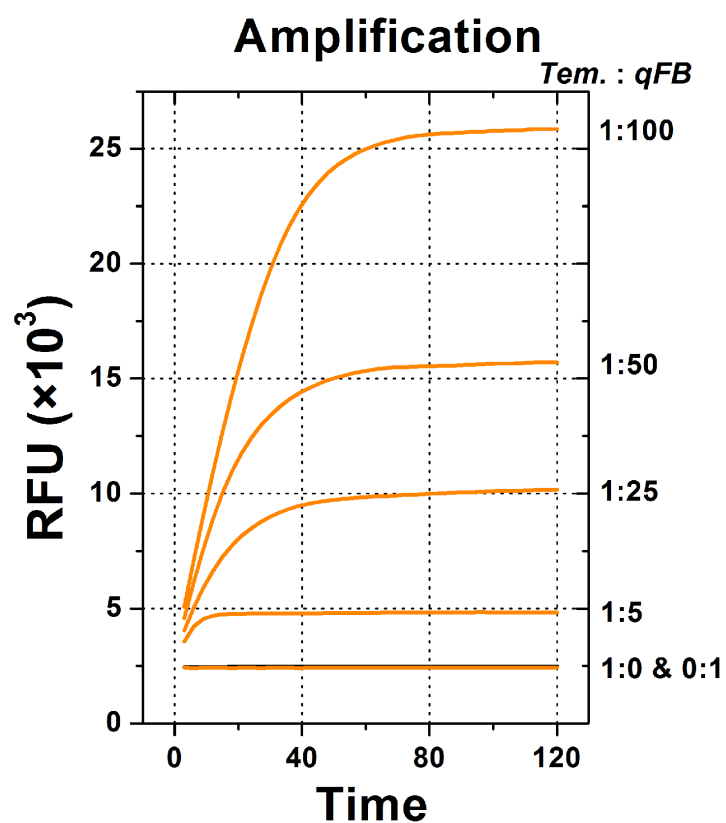
Figure S7. Real-time monitoring of the nicking enzyme reaction. The reaction mixture contains 10 nM template repeats (Tem.) and 0, 50, 250, 500, 1000 nM qFBs as the substrates, respectively. The concentration ratios between *Tem.* and *qFB* are shown in right part of the figure.
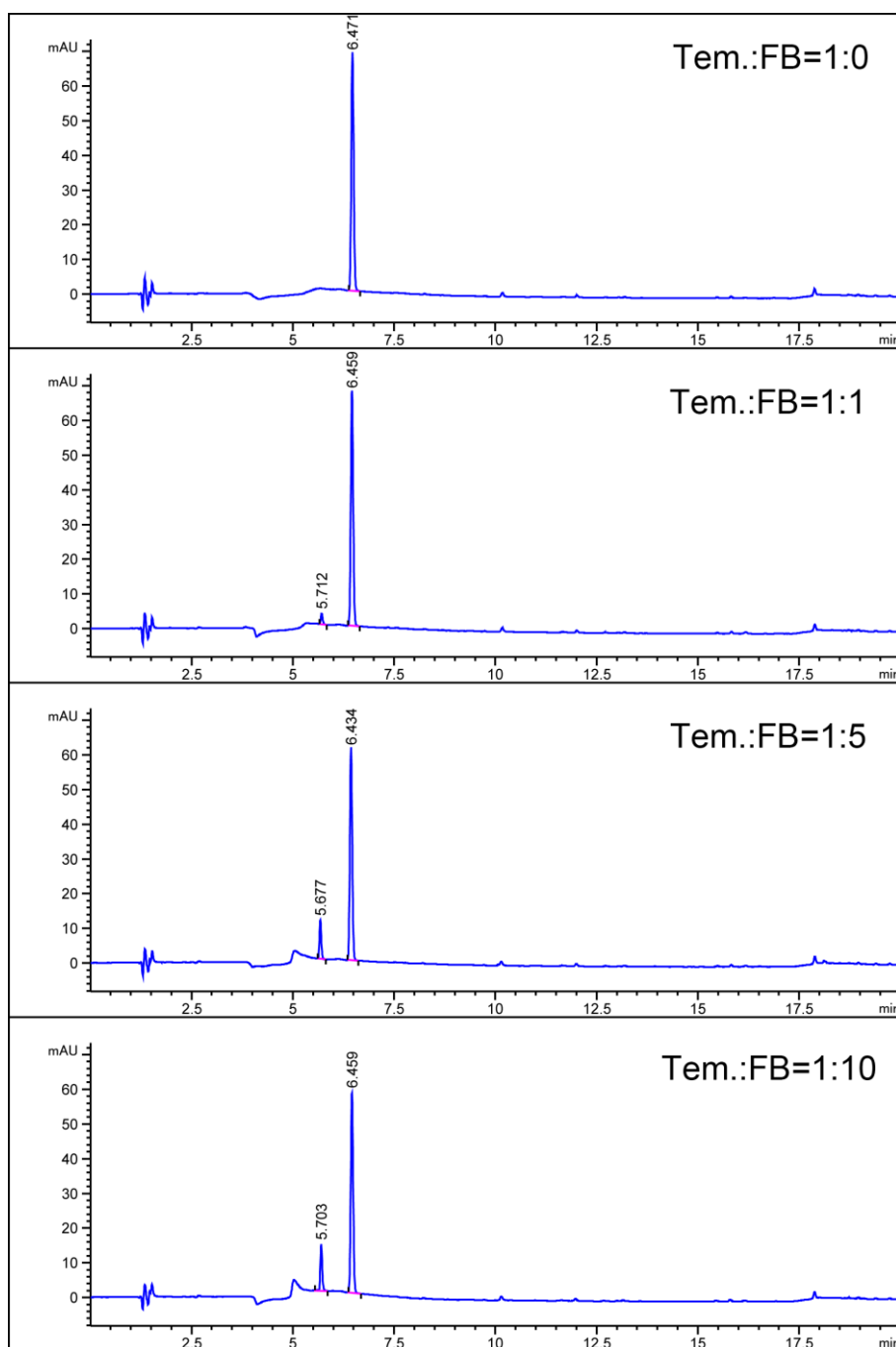
Figure S8. HPLC characterization of template-dependent cyclic degradation of fluorescence beacons (qFBs). Left peaks show short oligonucleotides generated from the degradation reaction, with a short retention time. Right peaks show long oligonucleotides Tem. and qFB, with a longer retention time. From top to down, the concentration of products increases with the concentration of the qFB, UV 254 nm.
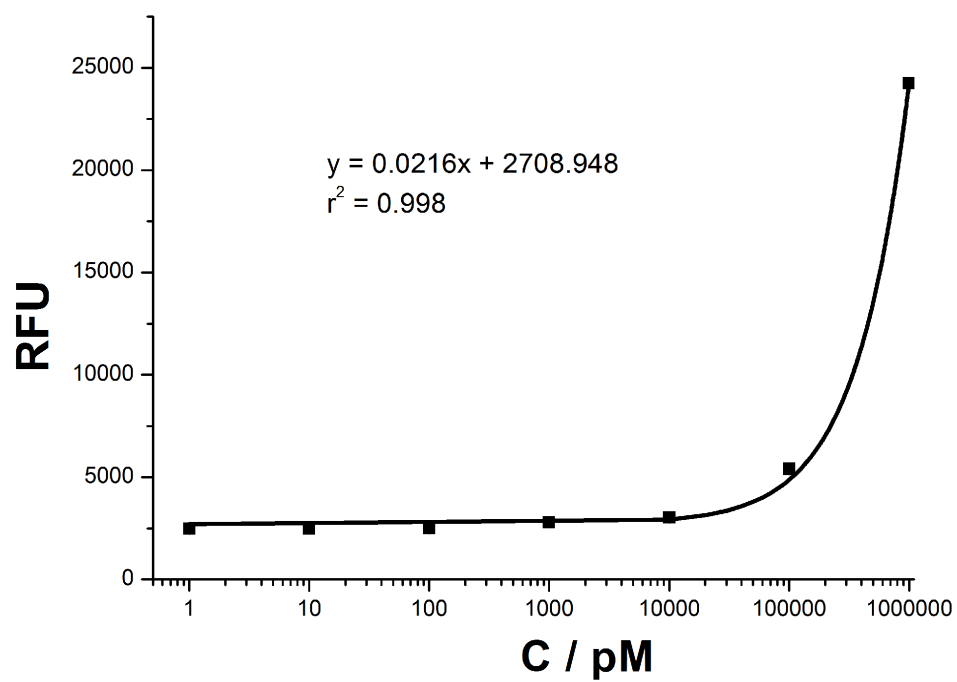
Figure S9. Calibration curve of the fluorescent signals were generated by digesting different concentrations of qFBs.
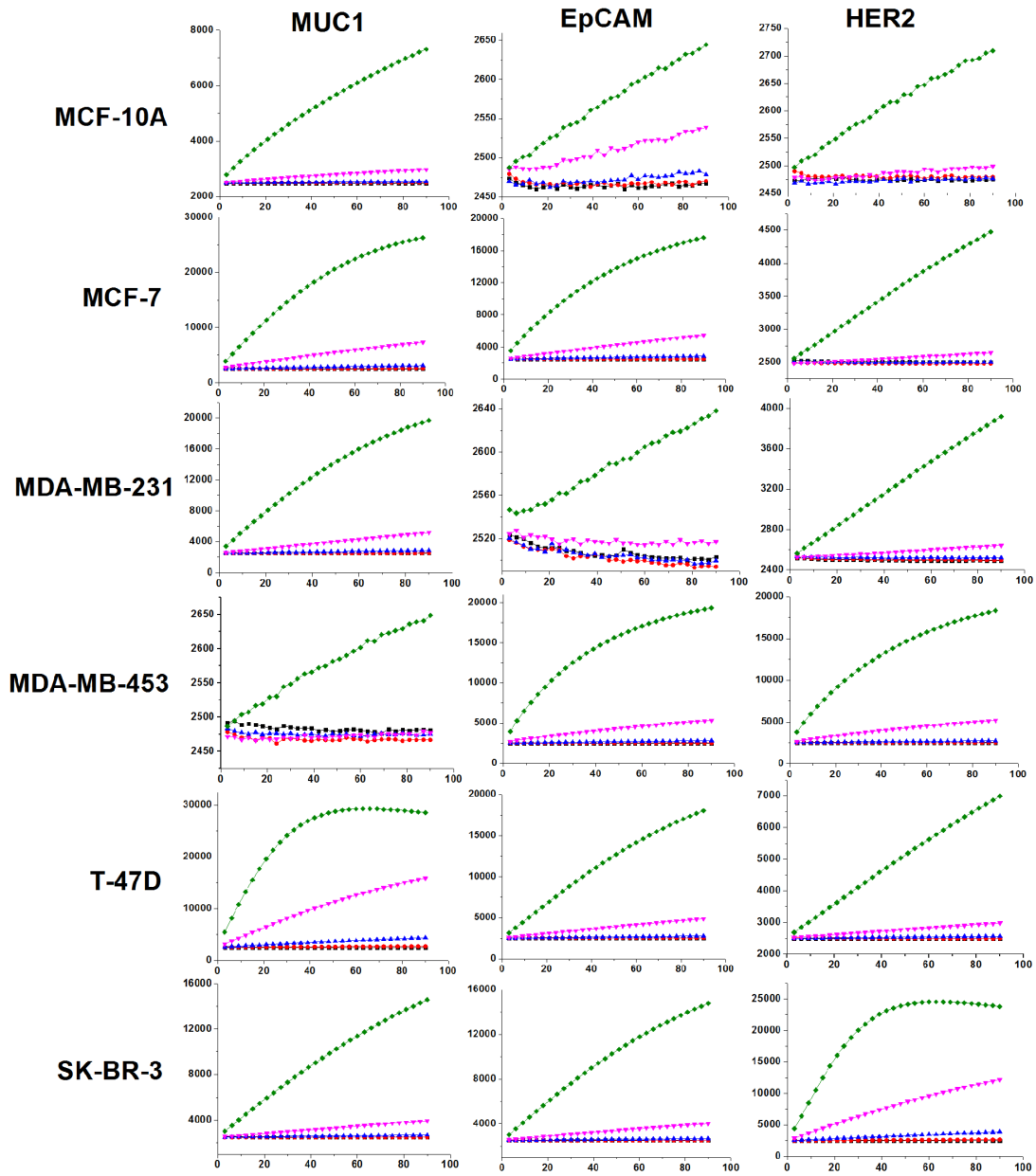
Figure S10. Profiling PMPs on different breast cell lines. For each of the cell line, various numbers of cells (0, 1, 10, 100 and 1000, corresponding to curves from bottom to top) were used to perform *is*RTA. The quantitation results for MUC1, EpCAM and HER2 protein biomarkers were shown in Figure 5.

Table S1. DNA oligonucleotide sequences used in experiments.

DNA	Sequence (5' to 3')	Modification
splint DNA	GAGAGGGTTGAGGATAGGACATT	none
pre-cDNA1	TCAACCCTCTCGTTTCTTGCTGAGGCTGTTAT CTTGCTGAGGCTGACGCACCTTGCTGAGGCT GCCTGAATGTCCTATCC	5′-PO$_4$
pre-cDNA2	TCAACCCTCTCGTTTTGTCATATAGTGTCTTTA TTGTCATATAGTGTCTACGCACTGTCATATAGT GTCTCCTGAATGTCCTATCC	5′-PO$_4$
pre-cDNA3	TCAACCCTCTCGTTTACGCAGTATTATGGTTAT ACGCAGTATTATGGACGCACACGCAGTATTAT GGCCTGAATGTCCTATCC	5′-PO$_4$
AP$_1$	GCAGTTGATCCTTTGGATACCCTGGTTTTTTT CAGCCTCAGCAAG	5′-NH2
AP$_2$	CACTACAGAGGTTGCGTCTGTCCCACGTTGT CATGGGGGGGTTGGCCTGTTTTTTTTTTAGACA CTATATGACA	5′-NH2
AP$_3$	GCAGCGGTGTGGGGGCAGCGGTGTGGGGGC AGCGGTGTGGGGTTTTTTTCCATAATACTGCG T	5′-NH2
iFB$_1$	CTTGCTGAGGCTG	5′-FAM
iFB$_2$	TGTCATATAGTGTCT	5′-AMCA
iFB$_3$	ACGCAGTATTATGG	5′-Cy3
qAP$_1$	GCAGTTGATCCTTTGGATACCCTGGTTTTTTT CAGCCTCAGCAAG	5′-NH2
qAP$_2$	CACTACAGAGGTTGCGTCTGTCCCACGTTGT CATGGGGGGGTTGGCCTGTTTTTTTTTTCAGCC TCAGCAAG	5′-NH2
qAP$_3$	GCAGCGGTGTGGGGGCAGCGGTGTGGGGGC AGCGGTGTGGGGTTTTTTTCAGCCTCAGCAA G	5′-NH2
qFB	TAGCTT<u>GCTGAGG</u>CTG	5′-ROX; 3′-BHQ2

Table S2. PPi assay to test RCA reaction efficiency.

C_{AP}/pM	Fluorescence/ RFU	C_{PPi}/mM	DNA length *per* amplicon/nt	Tem. repeats *per* amplicon/copies	Amplification magnitudes
10	667±36	0.56	55900	2096	3.32
100	977±47	5.76	57600	2160	3.33
1000	1485±80	18.29	18290	686	2.84

Table S3. Fluorescent signal calibration to test isRTA reaction efficiency.

C_{AP}/aM	Fluorescence/RFU	C_{qFB}/pM	Amplification magnitudes
10^2	2480±4	8.16	4.91
10^3	2498±7	16.80	4.83
10^4	2529±8	690.13	4.84
10^5	2841±12	5984.31	4.78
10^6	7172±26	194019.03	4.69
10^7	24324±52	963037.88	4.98
10^8	24848±114	986506.36	3.99

Table S4. Buffers used in the enzyme reaction activity tests.

Buffers	Components	RTA reaction activity (%)
H_2O	—	0
PRB	50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 4 mM DTT, pH 7.5 @ 25℃	100
NRB	20 mM Tris-HAc, 50 mM KHAc, 10 mM $Mg(HAc)_2$, pH 7.9 @ 25℃	59
PBS	135 mM NaCl，4.7 mM KCl，10 mM Na_2HPO_4，2 mM NaH_2PO_4, pH 7.4 @ 25℃	0
Reaction buffer	50 mM Tris-HCl, 85 mM NaCl, 10 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 4 mM DTT, pH 7.5 @ 25℃	96

References:

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