Ultrasensitive Quantification of Tumor mRNAs in Extracellular Vesicles with Integrated Microfluidic Digital Analysis Chip

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SUPPORTING INFORMATION

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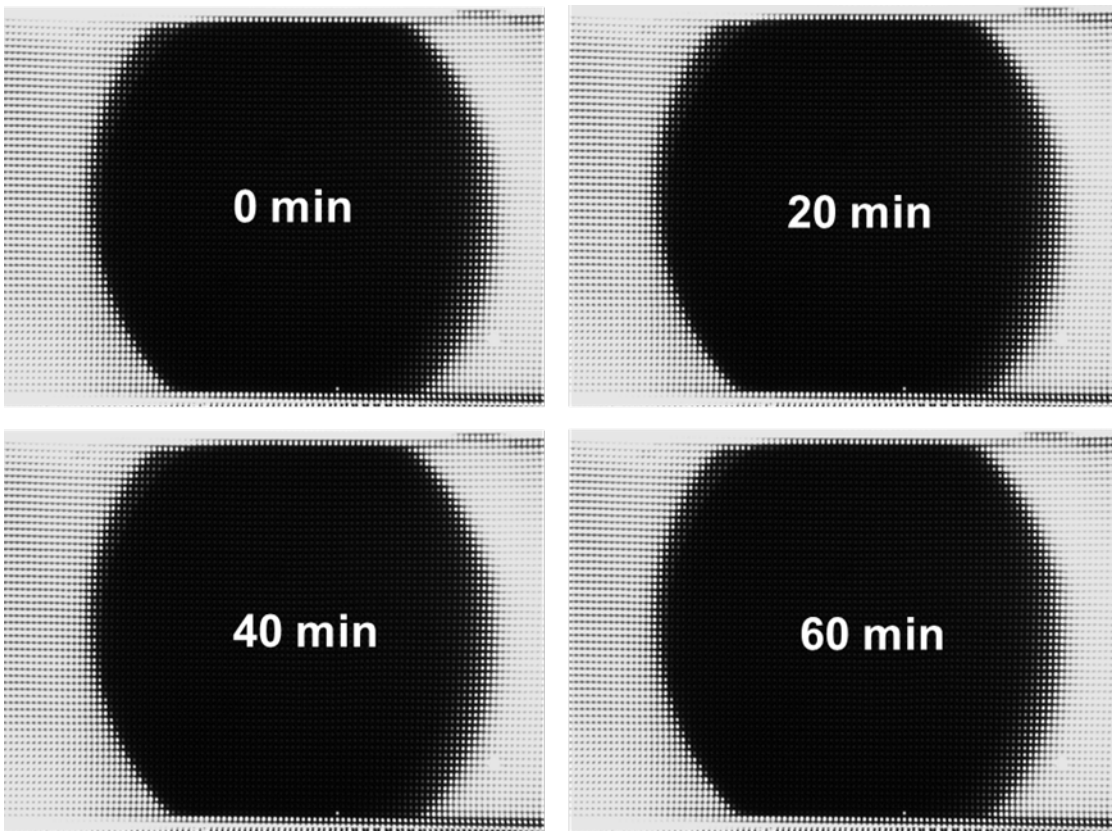

Figure S1. Characterization of sealing performance. No diffusion of fluorescent dyes into the photobleached area was observed over 60 min after sealing with the mechanical press.

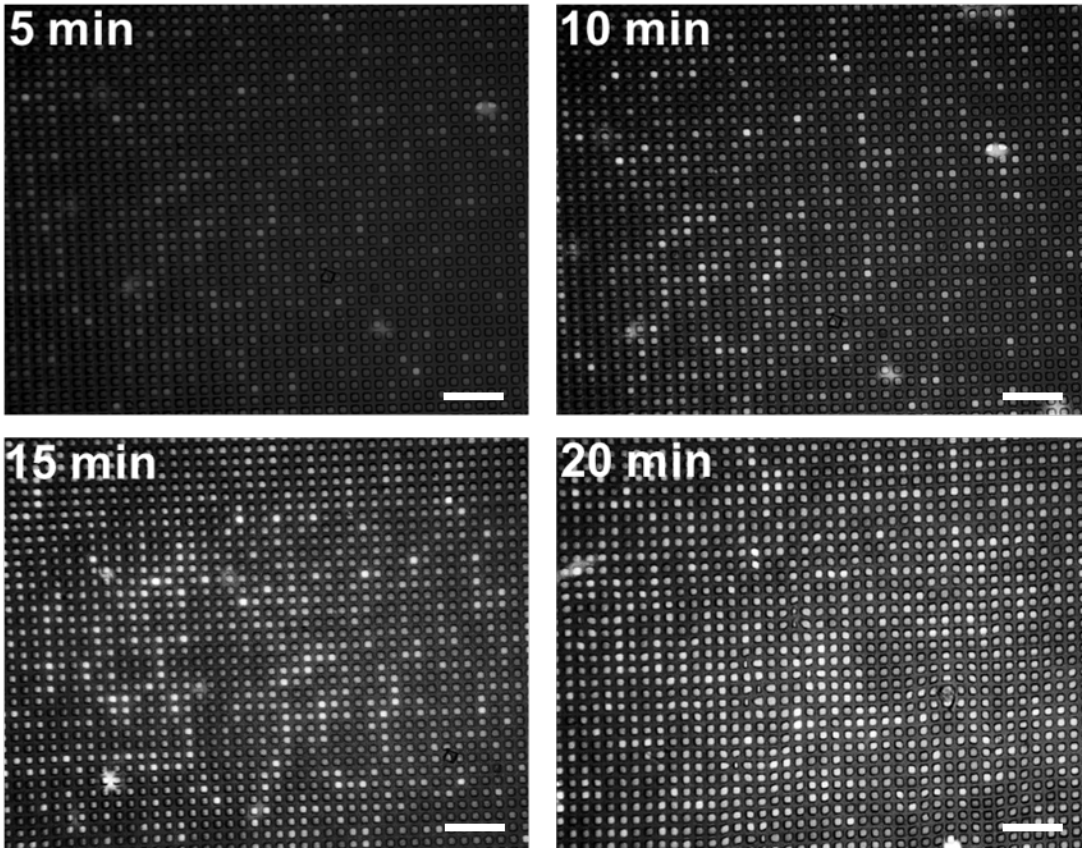

Figure S2. Representative fluorescence images for monitoring the signal intensity of individual fL reactions over 20 min (scale bar is 100 μm).

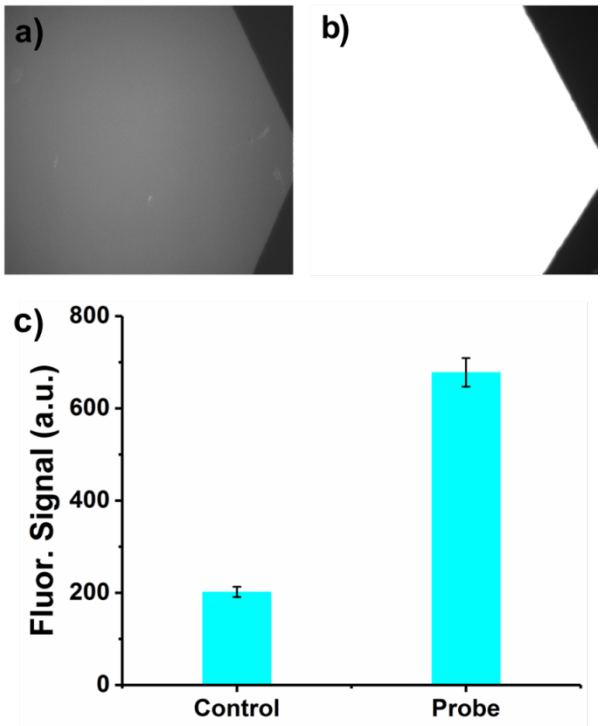

Figure S3. Fluorescent images (a) before and (b) after immobilization of 3'-FAM labelled capture probes on APTES and glutaraldehyde treated glass slide, and (c) corresponding intensity of fluorescent signals.

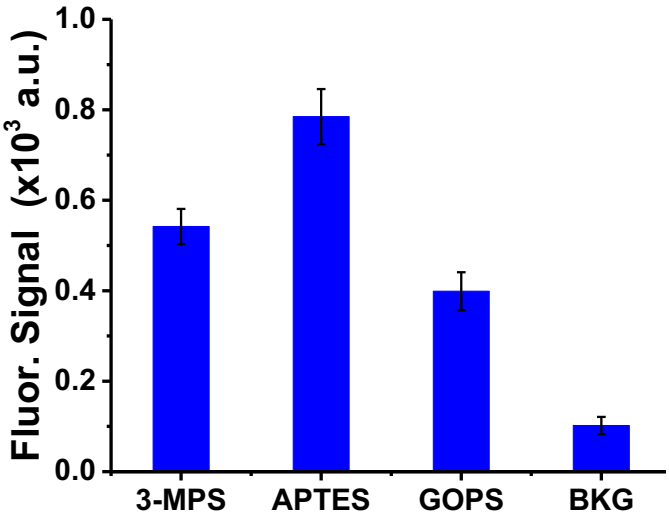

Figure S4. Optimization of surface treatment of glass substrate. The protocols of surface modification were adopted from the prior report.[1]

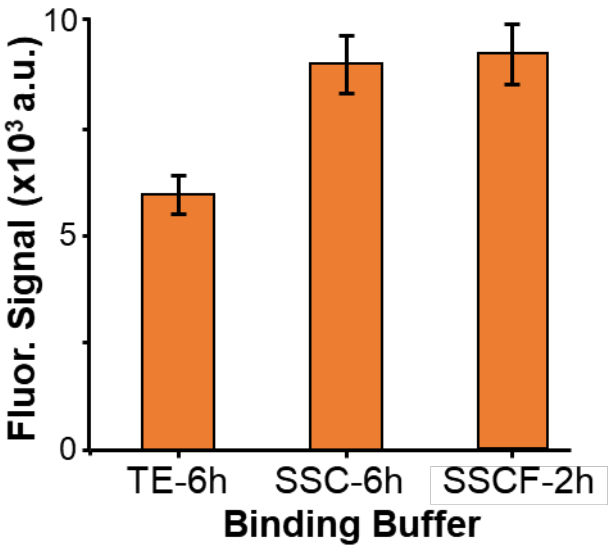

Figure S5. Optimization of the binding buffer and incubation time for the double hybridization assay.

Table S1. Probability Calculated by Poisson Distribution.

Average rate of occurrence, λ	Number of occurrences, *k*	Probability *P(X=k)*. %
1	0	36.8
	1	36.8
	≥2	26.4
0.1	0	90.5
	1	9.05
	2	0.905
0.01	0	99.0
	1	0.990

Table S2. Sequences of Capture and Detection Probes for *GAPDH* and *EWS-FLI* type 1 and type 3 Transcripts.

Probes	Oligo Sequence	Location in Fusion mRNA
GAPDH		
Capture probe CP1	5'-NH_2-C_{12}-AGGTCCACCACTGACACGTTG-3'	
Detection probe DP1	5'-GCAGTGGGGACACGGAAGGCC-TEG-biotin-3'	
Detection probe DP2	5'-TGTAGTTGAGGTCAATGAAGGG-TEG-biotin-3'	
***EWS-FLI* Type 1**		
Capture probe CP2	5'-NH_2-C_{12}-GCACTTGCGAATCTGCTTGA-3'	FLI1, exon 9
Detection probe DP3	5'-GCAACTCTTGTCCCAGTCCTC3'-TEG-biotin-3'	EWS, exon 1
Detection probe DP4	5'-CTGGATAAGCAGGCTGAGTG3'- TEG-biotin-3'	EWS, exon 5
***EWS-FLI* Type 3**		
Capture probe CP2	5'-NH_2-C_{12}-GCACTTGCGAATCTGCTTGA-3'	FLI1, exon 9
Detection probe DP5	5'-TGGGTCCACCAGGCTTATTG3'-TEG-biotin-3'	EWS, exons 9, 10
Detection probe DP6	5'-GGTGGTCCTGTCGGAATGAA3' -TEG-biotin-3'	EWS, exon 8

Table S3. Synthetic GAPDH Oligonucleotides Sequence.

Synthetic *GAPDH* oligonucleotides sequence
5'-CAAGGUCAUCCCUGAGCUGAACGGGAAGCUCACUGGCAUGGCCUUC CGUGUCCCCACUGCCAACGUGUCAGUGGUGGACCUGACCUGCCGUCU AGAAAAACCUGCCAAAUAUGAUGACAU-3'

Table S4. Common Types of PNET Fusion and Corresponding Genetic Breakpoints.

Fusion Type	Fusion Exons
EWS-FLI1 type 1	*EWS*(1-7) + *FLI*(6-9)
EWS-FLI1 type 2	*EWS*(1-7) + *FLI*(5-9)
EWS-FLI1 type 3	*EWS*(1-10) + *FLI*(6-9)
EWS-ERG	*EWS*(1-7) + *ERG*(6-10)

Table S5. Characterization of EVs Isolated from CHLA-9 and CHLA-258 Cells.

Sample	Concentration (/mL)	Mean diameter (nm)
CHLA-9 EVs	1.17×10^{12}	152.1
CHLA-258 EVs	2.08×10^{11}	127.3

REFERENCES:

1. Goddard, J.; Erickson, D., *Anal. Bioanal. Chem.* **2009,** *394* (2), 469-479.