SUPPORTING INFORMATION

Digital Quantification of miRNA directly in plasma using Integrated Comprehensive Droplet Digital Detection

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Table s1. Oligonucleotides used in this work.

Name	Sequence
Let-7a	5’-UGA GGU AGU AGG UUG UAU AGU U-3’
Let-7b	5’-UGA GGU AGU AGG UUG UGU GGU U-3’
Let-7c	5’-UGA GGU AGU AGG UUG UAU GGU U-3’
EXPAR template for Let-7a detection	5’-A ACT ATA CAA CCT ACT ACC TCA AAC A*GA CTC* AAA CTA TAC AAC CTA CTA CCT CAA-P-3’
miR-39	5’-UCA CCG GGU GUA AAU CAG CUU G-3’
EXPAR template for miR-92a detection	5’-A CAG GCC GGG ACA AGT GCA ATA AAC A*GA CTC* AA CAG GCC GGG ACA AGT GCA ATA A-P-3’
miR-92a	5’-UAU UGC ACU UGU CCC GGC CUG U-3’

Table s2. Quantification of spiked miRNA in 100% plasma using the IC 3D.

This table represents the raw data of Figure 3d. Accuracy is defined as percent accuracy (%Acc),

$$\%Acc = \left(1 - \frac{|Ps - Ap|}{Ps}\right) \times 100$$

where '*Ps*' is defined as the number of spiked miRNA, and '*Ap*' is defined as the actually detected number of miRNA.

Percent relative standard deviation (%RSD) represents precision of detection of each measurement,

$$\%RSD = \frac{s}{\bar{x}} \times 100$$

Where '$\bar{x}$' is defined as mean and '*s*' is defined as standard deviation from each of measurements. The experiments were performed using 100 uL plasma sample for microencapsulation and the data was converted to copy number of miRNA in 1 mL of plasma.

Number of spiked miRNA	Number of detected miRNA			Mean	Standard deviation	Accuracy (%)	% RSD
	1st experiment	2nd experiment	3rd experiment				
0	0.00	0.00	0.00	0.00	0.00	100.00	NA
10	0.00	0.00	0.00	0.00	0.00	0.00	NA
50	90.00	60.00	60.00	70.00	17.32	60.00	24.74
100	136.36	72.73	218.18	142.42	72.92	57.58	51.20
500	392.20	392.20	798.17	527.52	234.38	94.50	44.43
1000	1033.25	783.85	1952.49	1256.53	615.49	74.35	48.98
5000	3872.37	4913.26	6734.82	5173.48	1448.86	96.53	28.01
10000	5625.54	7441.76	10811.04	7959.45	2631.23	79.59	33.06
50000	24760.41	45373.94	51773.40	40635.92	14116.02	81.27	34.74
100000	82186.32	92532.00	76054.49	83590.94	8328.07	83.59	9.96

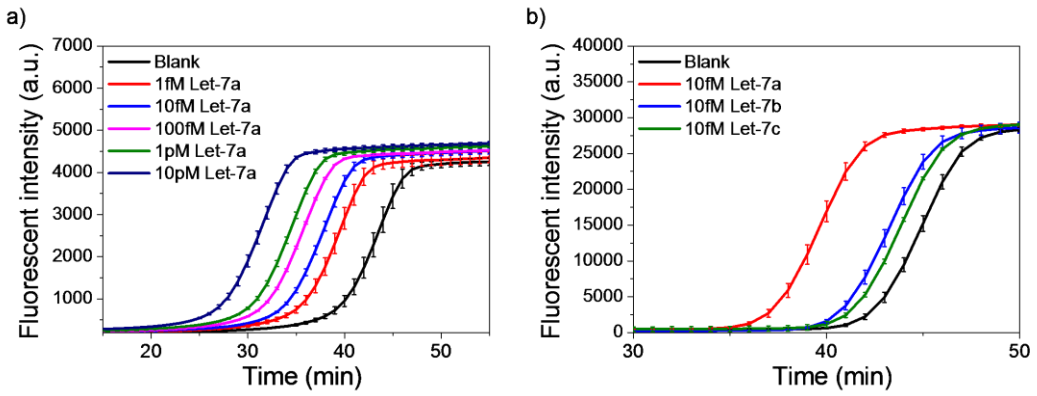

Figure s1. Exponential amplification reaction (EXPAR) for miRNA detection in reaction buffer (1X ThermoPol buffer, 0.5X NEBuffer 3.1). a) Real-time fluorescence measurement of EXPAR triggered by spiked 1 fM to 10 pM synthetic Let-7a miRNA. Error bar is based on triplicate experiments. Mean ± s.e.m. b) Real-time fluorescence measurement of EXPAR triggered by spiked 10 fM synthetic Let-7a, Let-7b and Let-7c. Blank is negative control without miRNA spike. Error bar is based on triplicate experiments. Mean ± s.e.m.

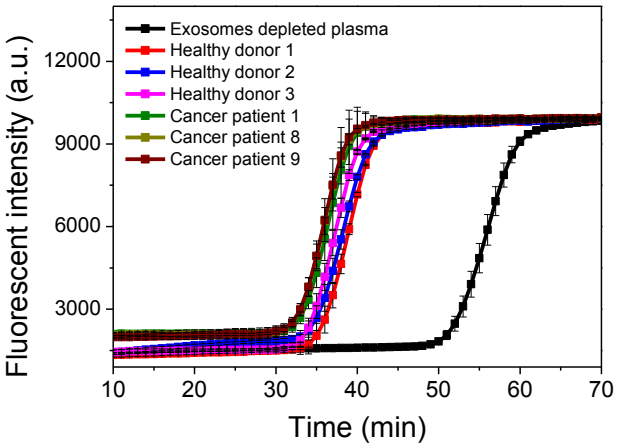

Figure s2. Real-time fluorescent measurement of miRNA-depleted plasma and colon cancer patient and healthy donor plasma samples using EXPAR in bulk. miRNA-depleted plasma control is from healthy donor sample. Error bar is based on triplicate experiments. Mean ± s.e.m.

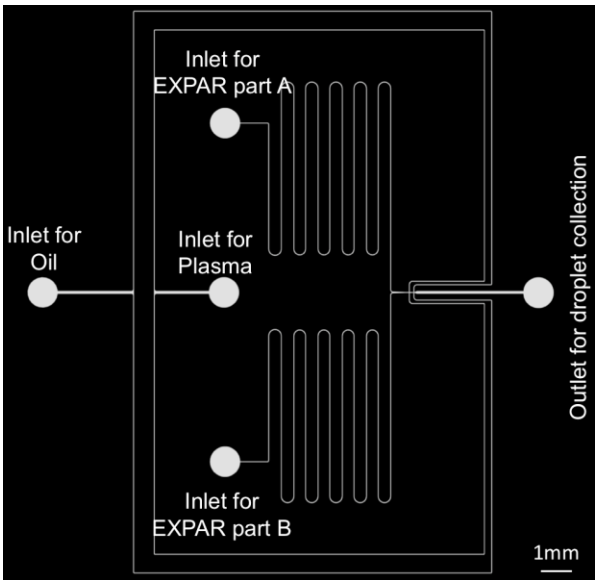

Figure s3. The CAD design of the microfluidic device for microencapsulation of the plasma.

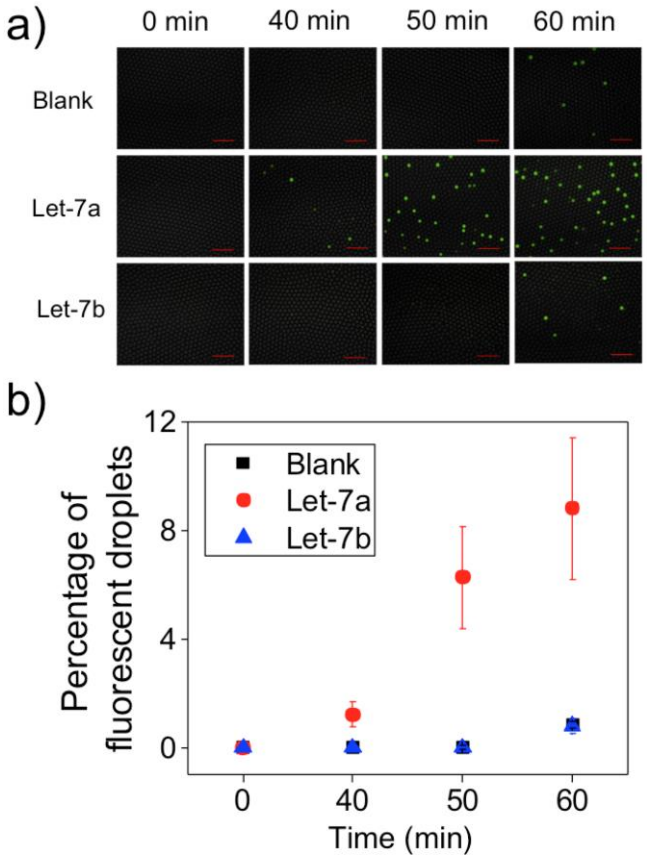

Figure s4. Kinetics study of digital droplet EXPAR. a) Representative fluorescent microscope images for the kinetics study of droplet single miRNA EXPAR. The bulk concentration of spiked miRNA before encapsulation was 10 fM which translates to 0 or

1 miRNA per droplets after encapsulation. The images at time points < 40 min are not shown because there are few fluorescent droplets in all cases. Scale bar: 200µm. b) Normalized detected fluorescent droplets over time. Droplets were imaged every 10 minutes while incubating at 55°C using a fluorescence microscope. The number of fluorescent droplets was analysed from microscopic images manually. Error bar is based on triplicate experiments where 2400 droplets were analysed in each quantification. Mean ± s.d., n=3.

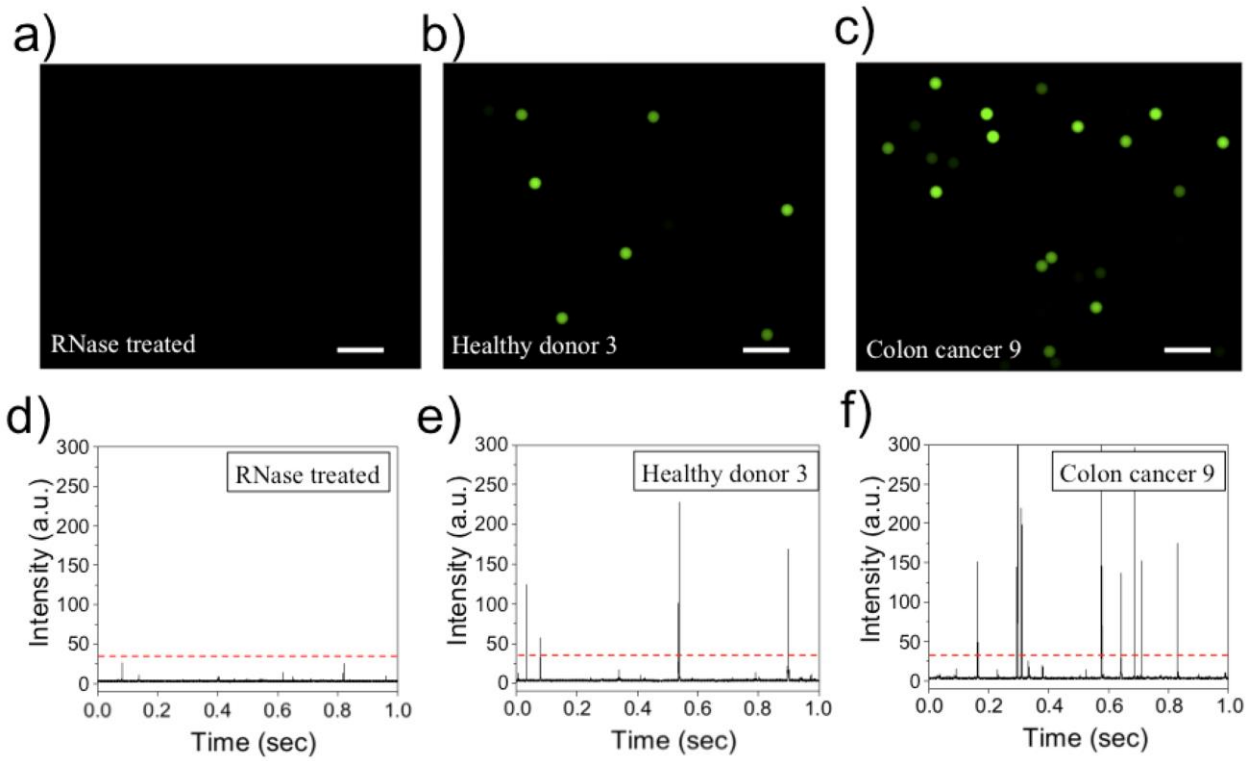

Figure s5. Let-7a was analyzed by droplet EXPAR from RNase treated-healthy donor plasma (a,d), Healthy donor plasma (b,e), and colon cancer plasma (c,f). (a, b, and c) are representative fluorescence microscopy images while (d, e, and f) are time trace of fluorescence intensity profiles of droplets from the 3D particle counter. EXPAR assay time is 50 min. Scale bar: 100µm. Threshold was obtained by 'Three sigma rule'.

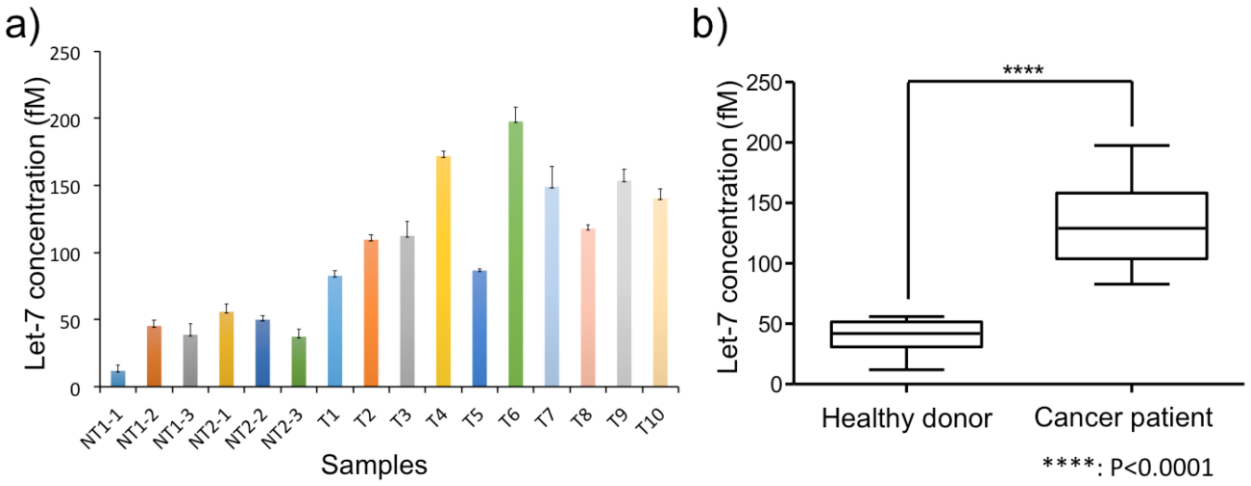

Figure s6. Quantification of Let-7a in healthy donors and cancer patients' plasma using RT-qPCR. a) Let-7a concentration results are shown as mean ± s.e.m. of triplicate experiments. NT: non-tumor; T: tumor. b) Comparison between control (healthy donor) and cancer patient plasma. P value < 0.0001 (student T test).

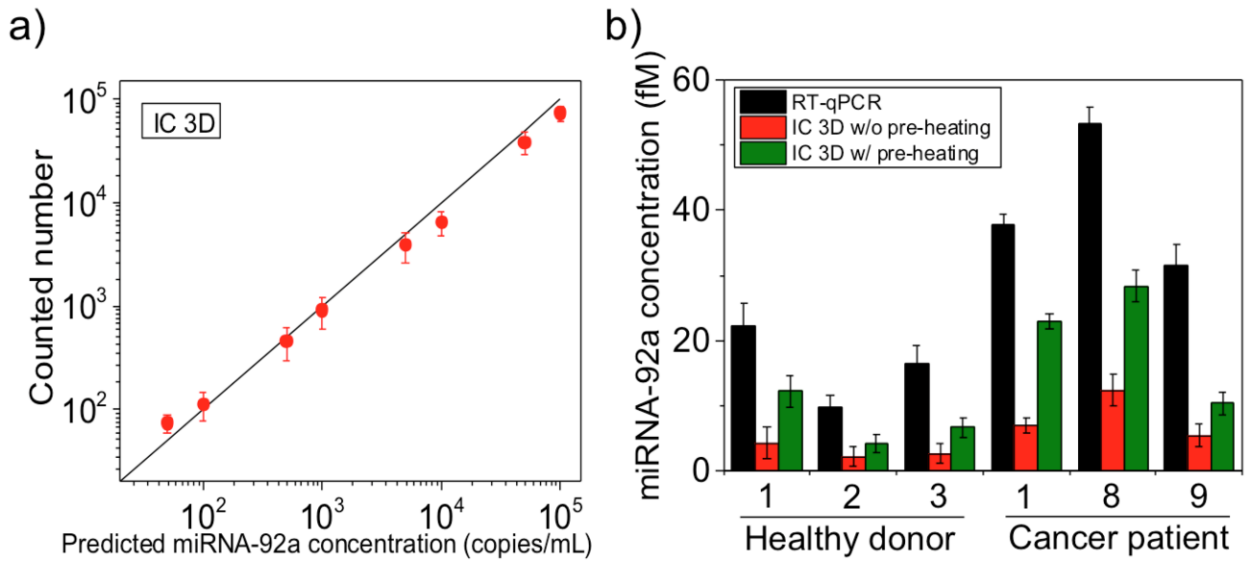

Figure s7. Quantification of miRNA-92a in healthy donors and cancer patients' plasma. a) Actually counted Let-7a number using IC 3D (y axis) vs. spiked Let-7a concentration (x axis) in miRNA depleted plasma. Error bar is based on triplicate experiments. Mean ± s.e.m. n=3. B) miRNA-92a concentration quantification in 3 healthy donor plasma samples and 3 colon cancer patient plasma samples detected by RT-qPCR and IC 3D_EXPAR. Error bar is based on triplicate experiments. Mean ± s.e.m. n=3.