Supplementary Section to

The fabrication of 1,2-dicarbonyl compound-caging isothermal exponential amplification strategy and its application in the highly sensitive detection of tumor exosomal miRNA

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Table S1 The RNA and DNA sequences used in this study.

Name	Sequence (5'- to 3'-)
X'-X'/CS16	AACTATACAACCTACTACCTCAAACAGACTCAAACTATACAA CCTACTACCTCAA-P
X'-Y'	CTCACGCTACAACAGACTCAAACTATACAACCTACTACCTCA A-P
Y'-Y'	CTCACGCTACGGACGACTCTCTCACGCTAC-P
X'-Y'-Y'	CTCACGCTACGGACGACTCTCTCACGCTACAACAGACTCAAA CTATACAACCTACTACCTCAA-P
NR154	CCTGCCTCACTTAAGACTCTCCTGCCTCAC-P
NR161	CCTGCCTCACTTAAGACTCTCCTGCCTCAC-P
NR278	CGACAACTTCTAATGACTCTCGACAACTTC-P
NR350	AATTCCCCTGGCTGGACTCTAATTCCCCTG-P
NR371	GAACCCCCGTTGCGGACTCTGAACCCCCGT-P
let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7c	UGAGGUAGUAGGUUGUAUGGUU
let-7e	UGAGGUAGGAGGUUGUAUAGU
let-7f	UGAGGUAGUAGAUUGUAUAGUU
let-7i	UGAGGUAGUAGUUUGUGCUGUU
T154	GTGAGGCAGG
T161	GGTGAGGTAC
T278	GAAGTTGTCG
T350	CAGGGGAATT
T371	ACGGGGGTTC
Hairpin target	TGAGGTAGTAGGTTGTATAGTT
Hairpin template	AACTATACAACCTACTACCTCATTCAGACTCAAACTATAC AACCTACTACCTCAAAGCAGCACCAAAAAGGTGCTGCAAA A-P
G3 target	CCCTCCCTTCCCGA
G3 template	TCGGGAAGGGAGGGAACAGACTCTTCGGGAAGGGAGGG-P
T38	ACGGGCAAGA
NR38	TCTTGCCCGTCGCCGACTCTTCTTGCCCGT-P
NR38-FAM	TCTTGCCCG/iBHQ1dT/CGCCGACTCTTCTTGCCCGT
NR38-BHQ1	TCTTGCCCG/i6FAMdT/CGCCGACTCTTCTTGCCCGT
primer	GTTATTGTGAGGTGATAGGTGATTGAGTAG
sanger template	CACTCTCACTACCTCTCAATACTCCATCTACCTCACATACT ACATCACTCCTATCTAACTACACTCATCATACATCACTGAC TCATCCTACTACTCAATCACCTATCACCTCACAATAAC
sanger primer	CACTCTCACTACCTCTCAATACTCC

Table S2 The efficiency of different 1,2-dicarbonyl compounds modified EXPAR.

1,2-dicarbonyl compound	modification time	ΔC_T increased times
glyoxal	10 min	5
methylglyoxal	10 min	2
phenylglyoxal	30 min	7
4-nitrophenylglyoxal	60 min	3
ninhydrin	120 min	9

Table S3 Comparison of different biosensors for the detection of miRNA.

Analytical method	Signal transduction	Dynamic range	LOD
Glyoxal caging EXPAR (This work)	Fluorescence	10 fM-10 pM	6.8 fM
HCR with DNAzyme[2]	Fluorescence	10 pM-10 nM	10 pM
Strip based on lateral transverse flow[3]	Fluorescence	＜100 nM	7.76 pM
MOs with microfluidic channel[4]	Fluorescence	Not given	25 nM
Graphene oxide nanoplates based RCA[5]	Fluorescence	2 pM-10 nM	2 pM
DNA-peptide probe assay[6]	LC-MS/MS	1 pM-100 nM	1 pM
Electrochemical strategy based on HCR[7]	Electrochemistry	1 pM-800 pM	1 pM
Allosteric effect of mismatched CHA for enzyme-free SPR[8]	SPR	5 pM-100 nM	1 pM

Table S4 Comparison of qRT-PCR and this method.

	This work	qRT-PCR
Sensitivity	10 fM-10 pM	1 fM-100 pM[9-10]
Specificity	Good	Good
Reproducibility	Good	Good
Reverse transcription	Don't need	Need
Total analysis time	1-2 h	2-3 h
Costs per run	~ 6 RMB	150 RMB

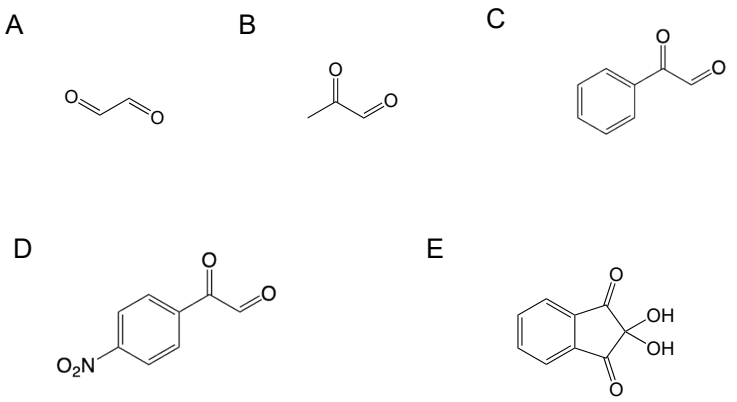

Fig. S1 The structural formulas of five 1,2-dicarbonyl compounds (A) glyoxal, (B) methylglyoxal, (C) phenylglyoxal, (D) 4-nitrophenylglyoxal, (E) ninhydrin, respectively.

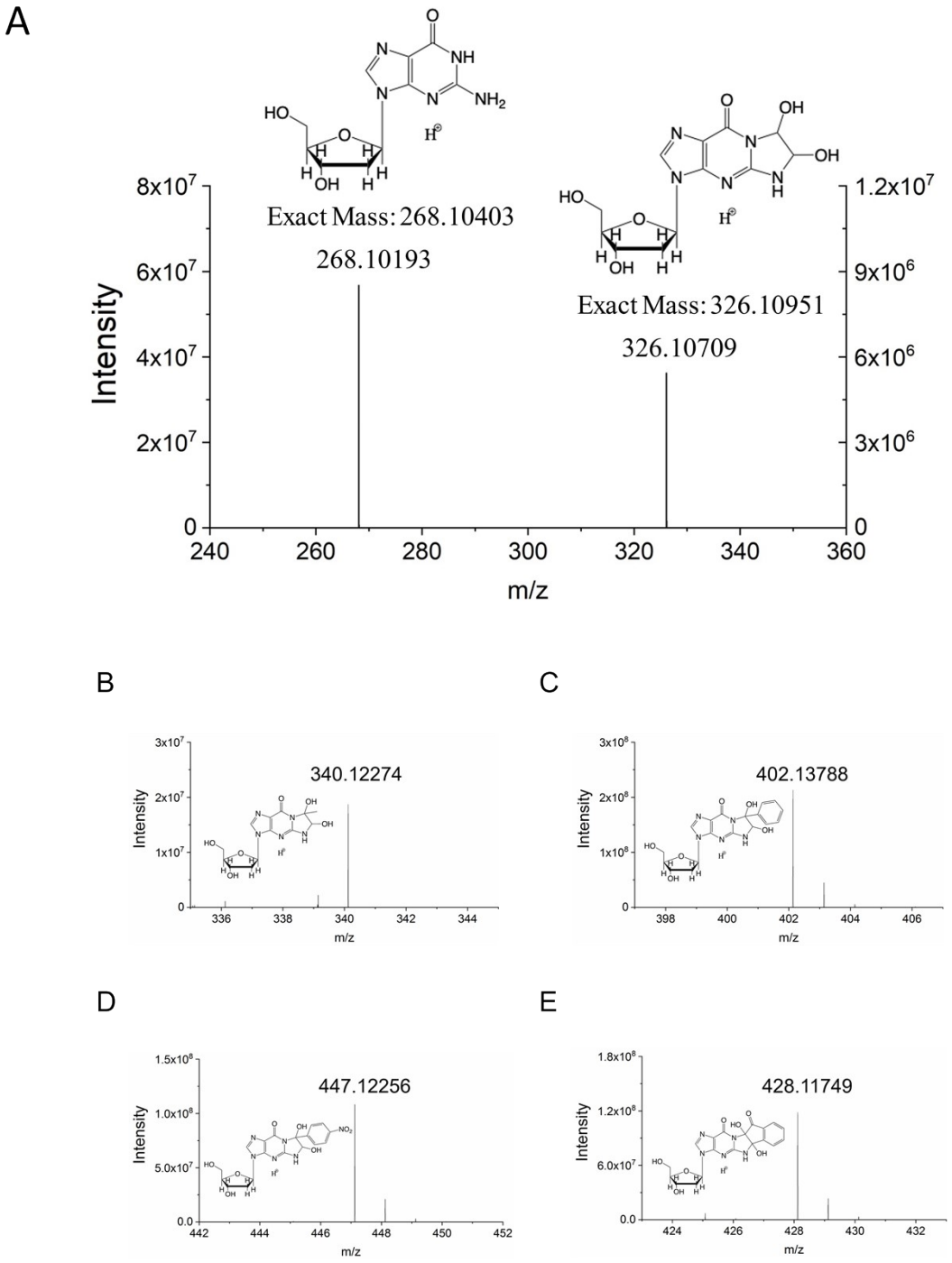

Fig. S2 The mass spectra of dG and its modified products. The modified 1,2-dicarbonyl compounds was (A) glyoxal, (B) methylglyoxal, (C) phenylglyoxal, (D) 4-nitrophenylglyoxal, (E) ninhydrin, respectively.

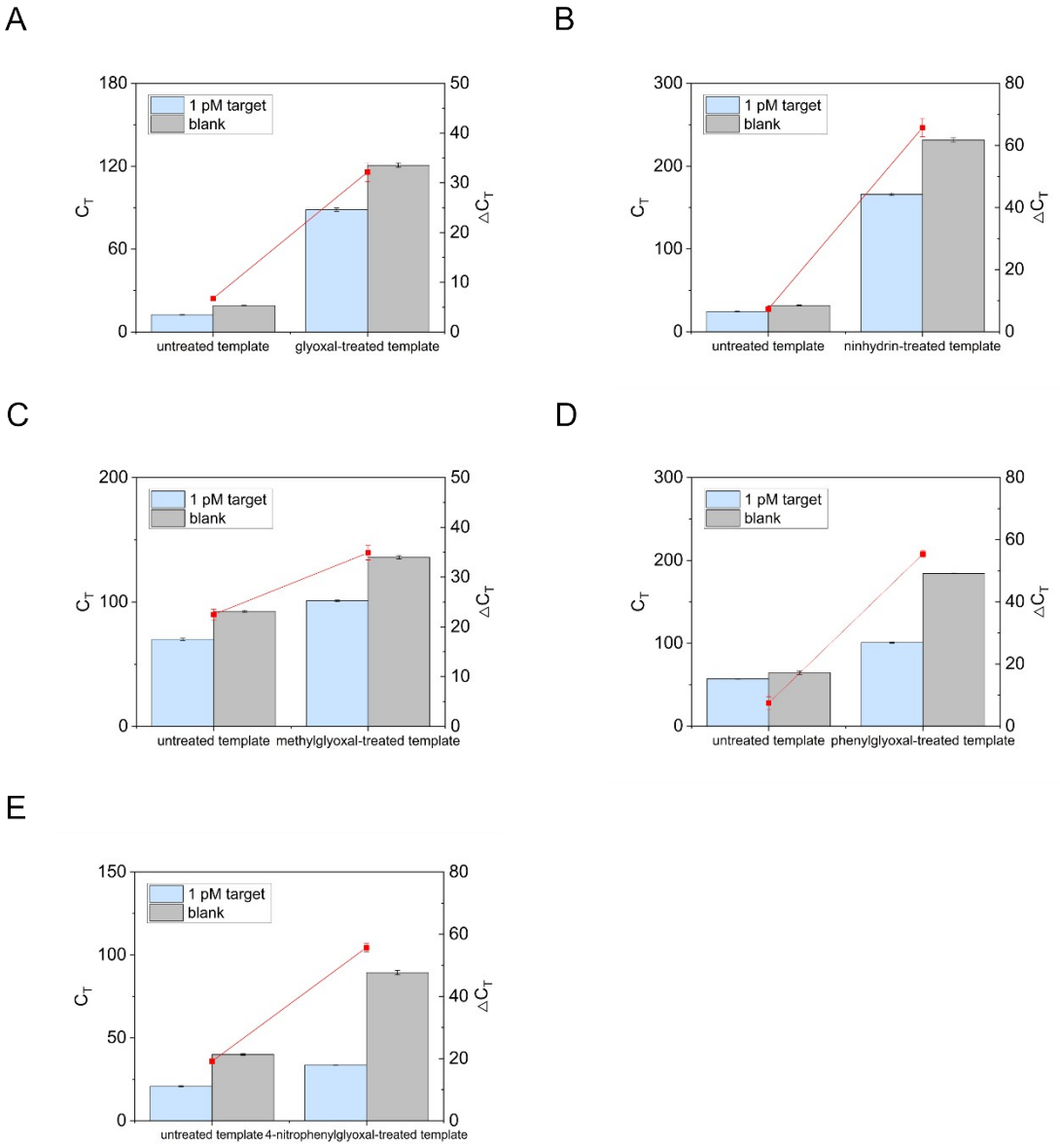

Fig. S3 Difference between the signal (1 pM target) and background (blank) with untreated and 1,2-dicarbonyl compound-treated template: (A) glyoxal; (B) ninhydrin; (C) methylglyoxal (D) phenylglyoxal; (E) 4-nitrophenylglyoxal.

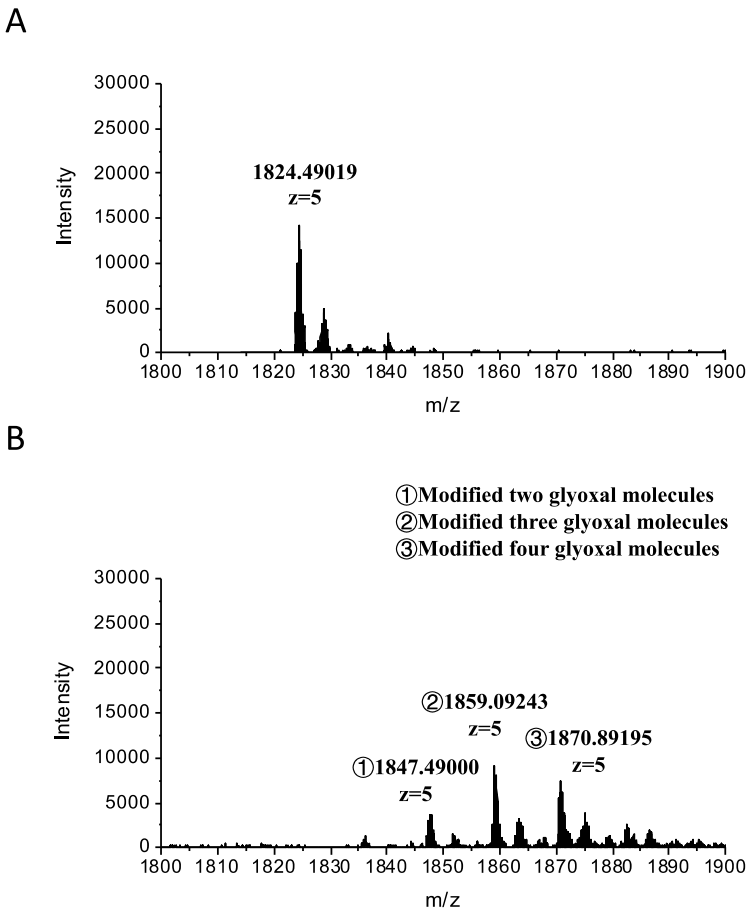

Fig. S4 Confirmation of the successful glyoxal modification of the template using high-resolution mass spectrometry. Figures S4-A and S4-B correspond to the mass spectra of the unmodified substrate chain and the modified substrate chain, respectively.

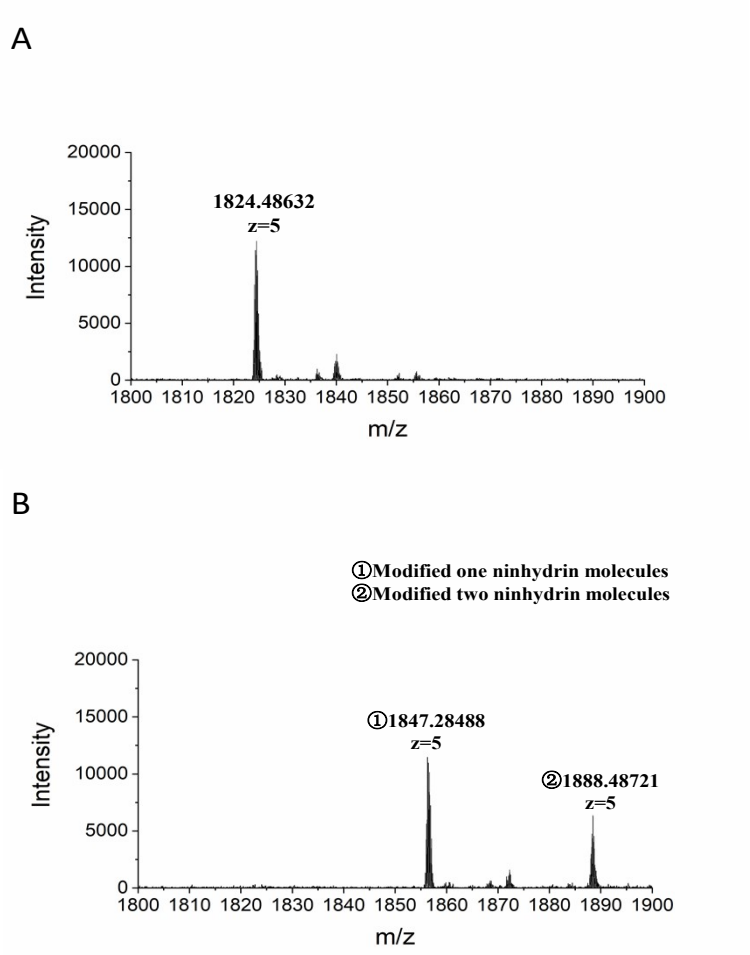

Fig. S5 Confirmation of the successful ninhydrin modification of the template using high-resolution mass spectrometry. Figures S5-A and S5-B correspond to the mass spectra of the unmodified substrate chain and the modified substrate chain, respectively.

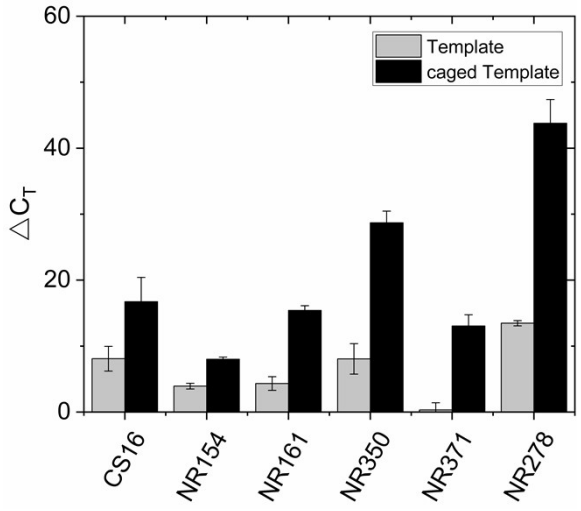

Fig. S6 Difference between the signal (1 pM target) and background (blank) with six kinds of uncaged and caged templates.

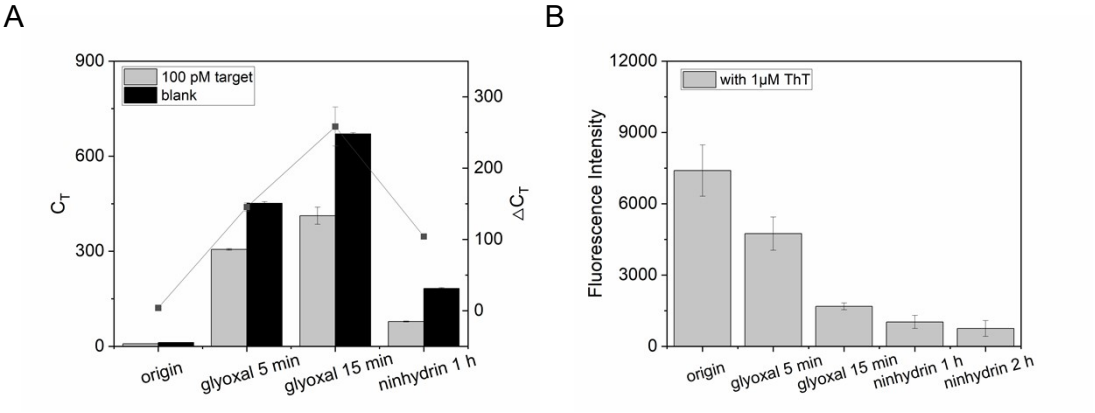

Fig. S7 The performance of G-triplex template before and after glyoxal and ninhydrin treatment (A) the differentiation between target signal and background in amplification reaction; (B) the change of fluorescent signal with ThT.

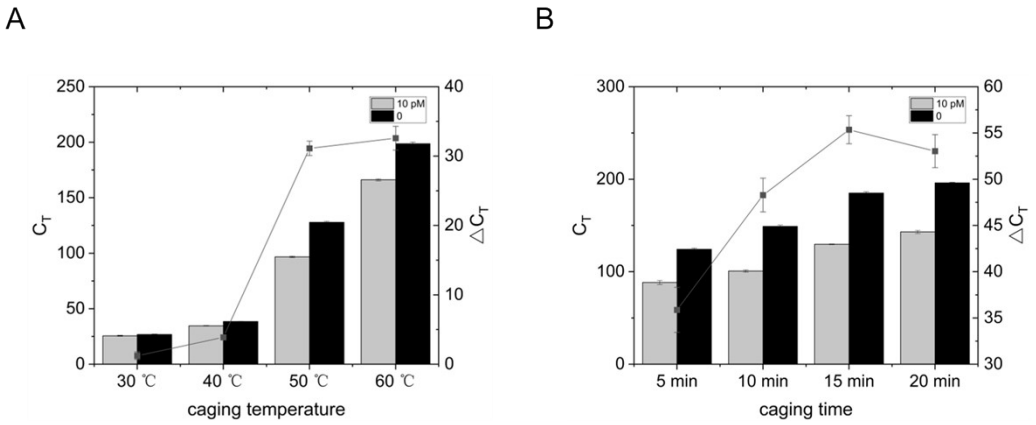

Fig. S8 Optimization of the reaction (A) temperature and (B) time of glyoxal with the template.

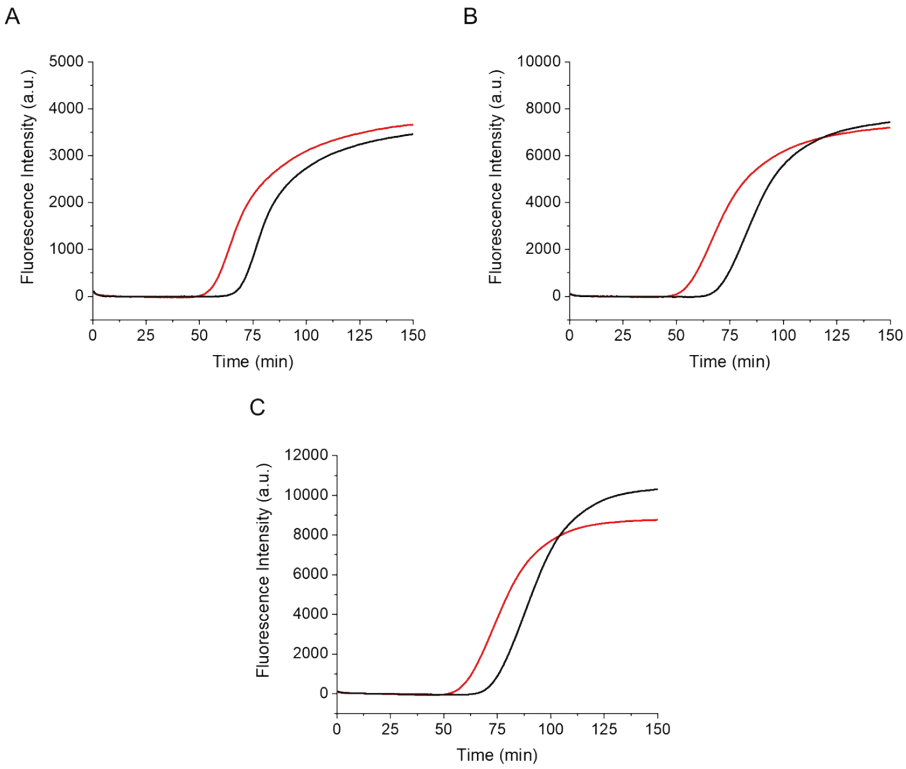

Fig. S9 Optimization of the concentration of glyoxal-modified template. The real-time fluorescence curves were produced by 10 pM let-7a miRNA and the blank with glyoxal treated EXPAR. The dilution ratio of the glyoxal modified template was (A) 5, (B) 2, (C) 0, respectively.

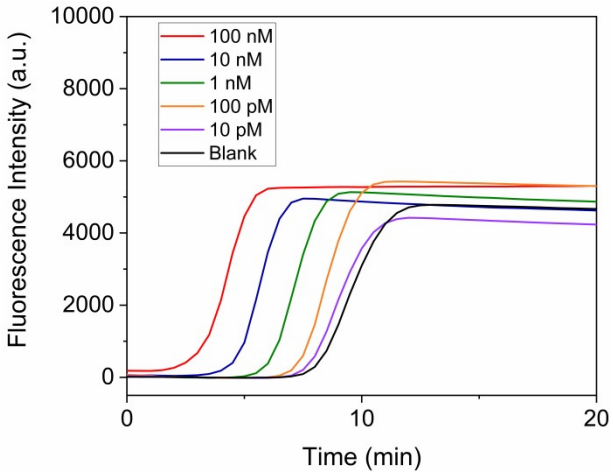

Fig. S10 Real-time fluorescence monitoring of standard EXPAR reaction with varying concentrations of target miRNA let-7a (10^{-11}-10^{-7} M).

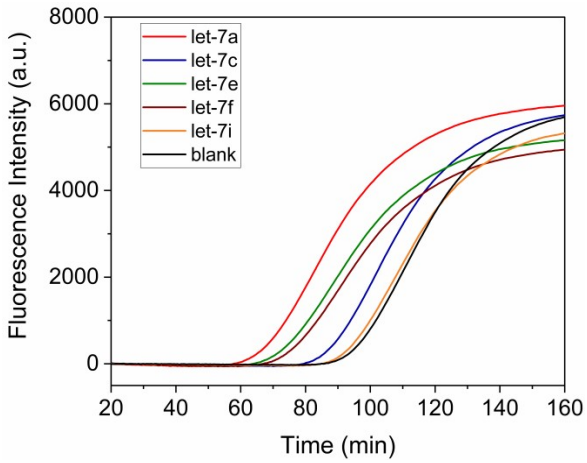

Fig. S11 Real-time fluorescence curves of the EXPAR triggered by miRNA lat-7a, let-7c, let-7e, let-7f, and let-7i.

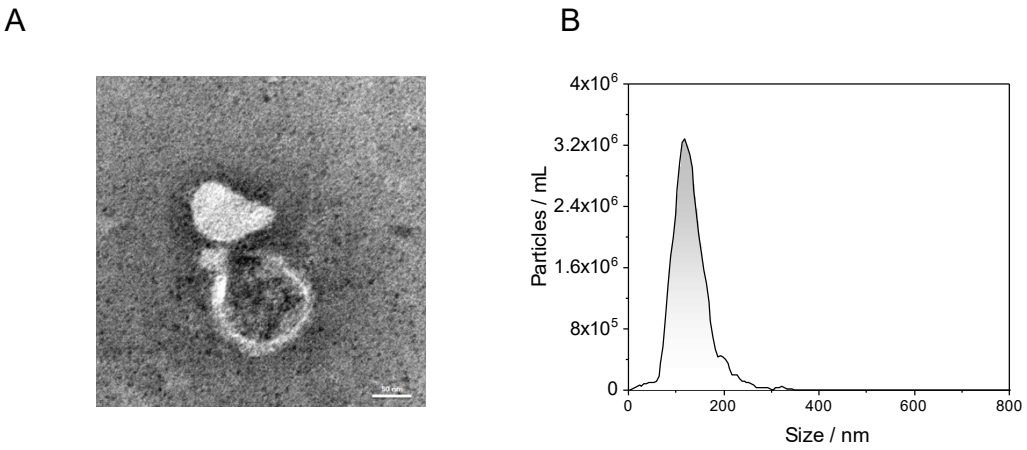

Fig. S12 Characterization of exosomes derived from MCF-7 cell cultures: (A) Transmission Electron Microscopy image of exosomes, scale bar = 50 nm; (B) Nanoparticle Tracking Analysis of exosomes.

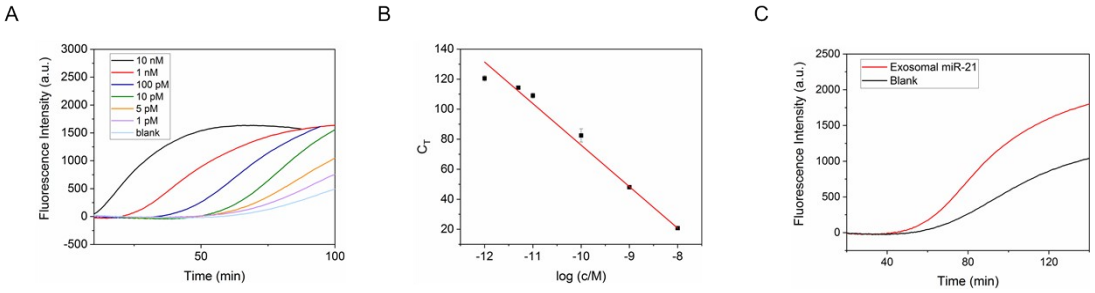

Fig. S13 Detection of miRNA-21 in MCF-7 exosomes: (A) Real-time fluorescence monitoring of 1,2-dicarbonyl compound-caging EXPAR reaction with varying concentrations of target miRNA-21; (B) A linear relationship between the C_T values and the logarithm (lg) of miRNA concentrations; (C) Real-time fluorescence curves for detection of exosomal miRNA-21 with 1,2-dicarbonyl compound-caging EXPAR.

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