Electronic Supplementary Information (ESI)

Two-Color-Based Nanoflares for Multiplexed MicroRNAs Imaging in Live Cells

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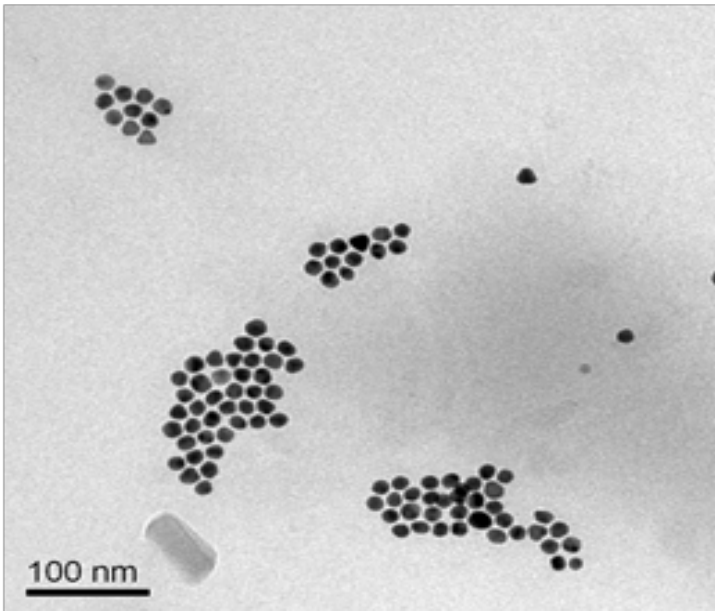

Fig. S1. TEM image of AuNPs

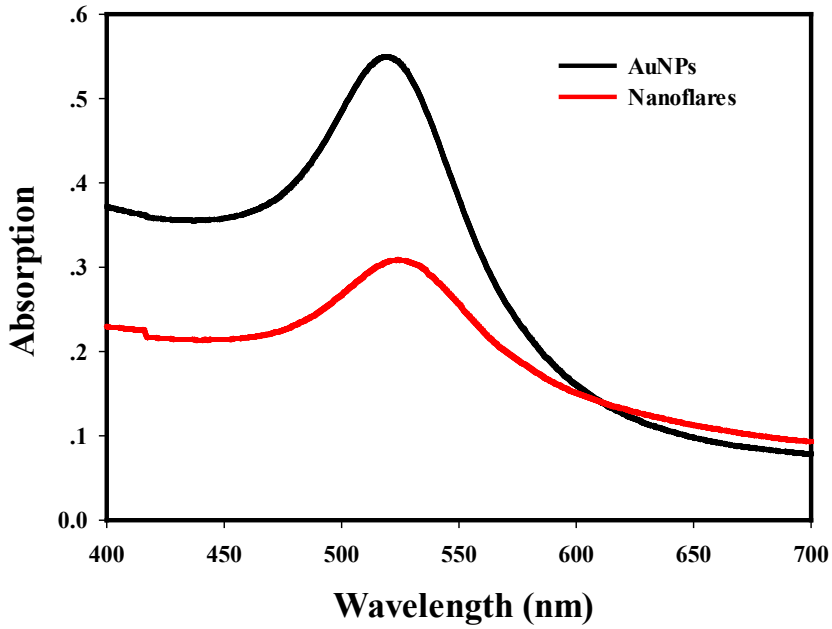

Fig. S2. Absorption spectra of AuNPs (black line) and DNA functionalized AuNPs (red line). The maximum optical absorption was shifted from 519 nm to 524 nm after modification.

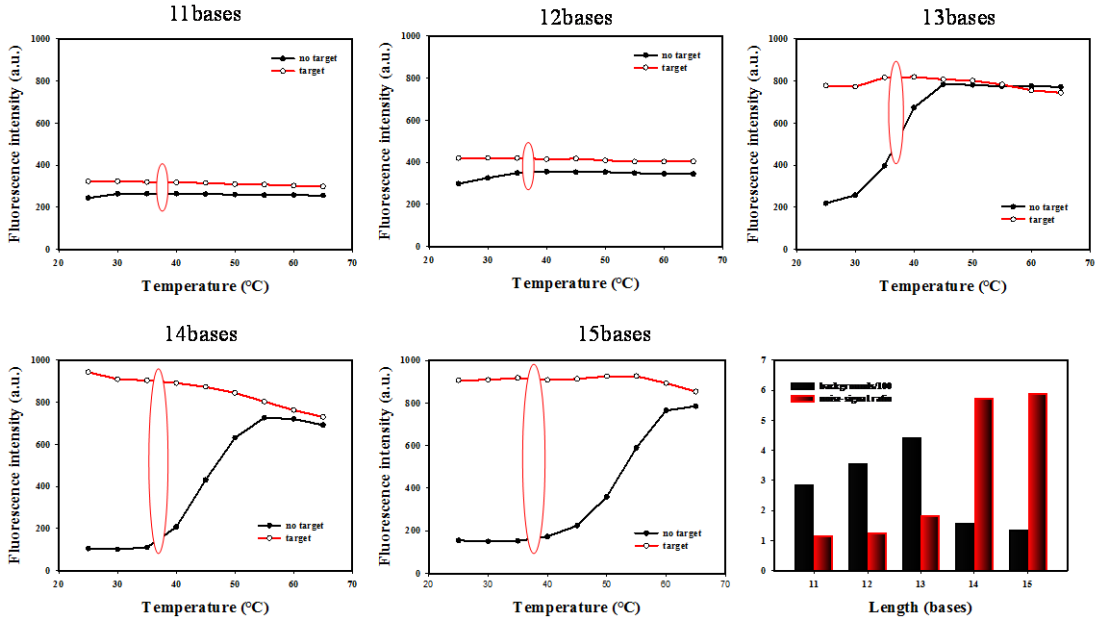

Fig. S3. The fluorescence recovery of different length flares (11, 12, 13, 14, 15bases) at various temperatures (red ring represents fluorescence recovery at 37 °C). The fluorescence of FAM was excited at 488 nm and measured at 520 nm.

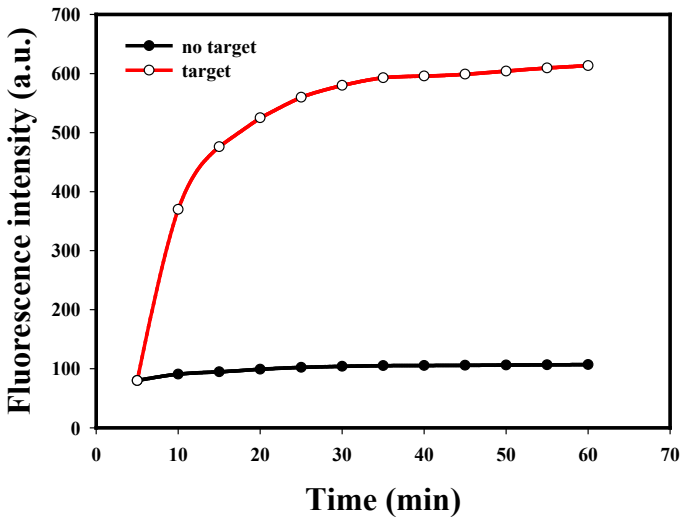

Fig. S4. the kinetic analysis of fluorescence intensity on the hybridization time for Nanoflares incubated with miRNA-21 target. The fluorescence of FAM was excited at 488 nm and measured at 520 nm.

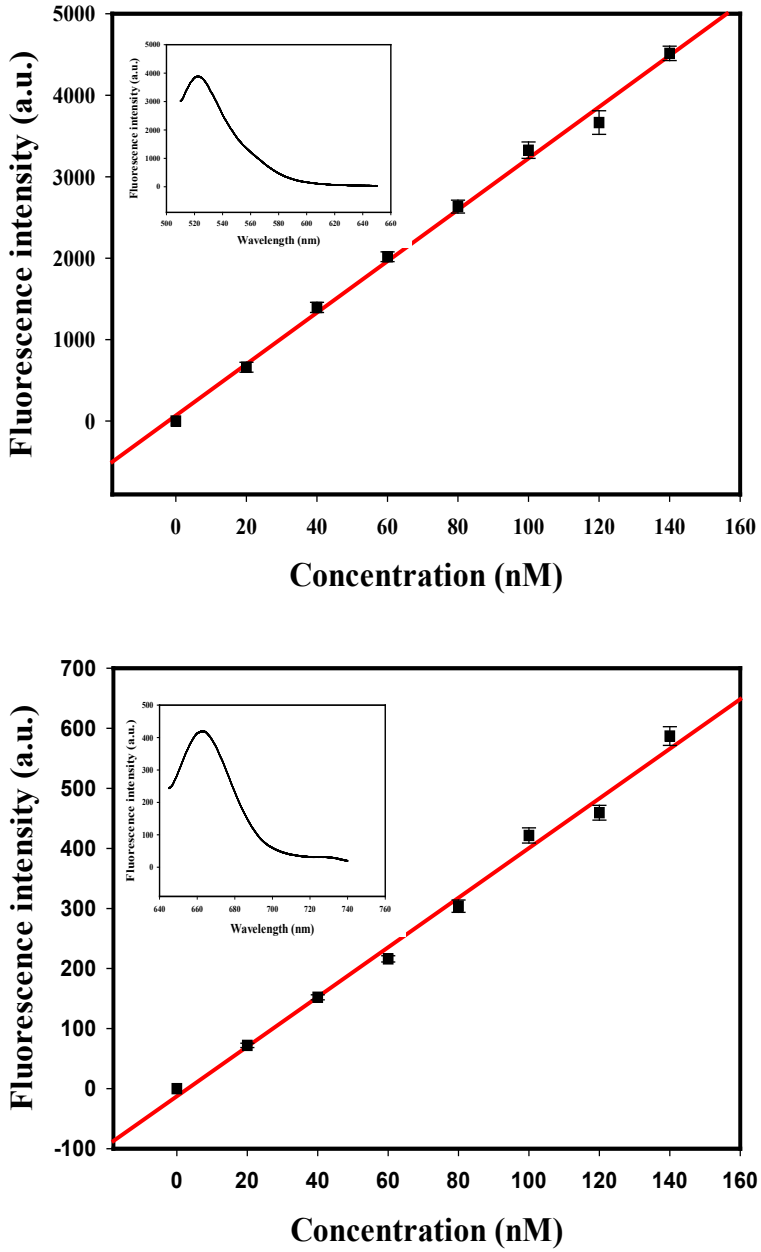

Fig. S5. Evaluation of Amounts of DNA Duplexes on each AuNP. Standard linear calibration curve of fluorescence signal against the concentration of FAM labeled flares-21 a) and Cy5 b) labeled flare.

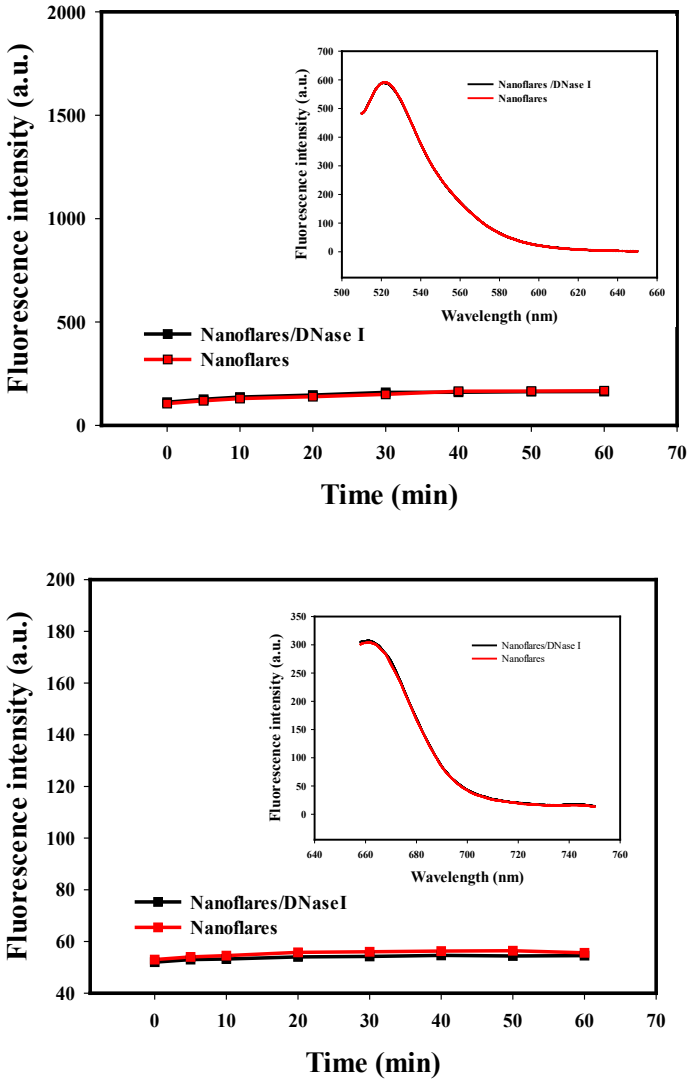

Fig. S6. Nuclease stability of the nanoflares in the presence or absence of DNase I. Fluorescence curves of the nanoflares (3 nM) without (a) or with (b) DNase I for 1h. Insets: fluorescence spectra after hybridization of the nanoflares with DNA targets in the absence (c) and presence (d) of DNase I. The above is miRNA-21 DNA target measured at 488 nm. The below is miRNA-141 DNA target measured at 635 nm.

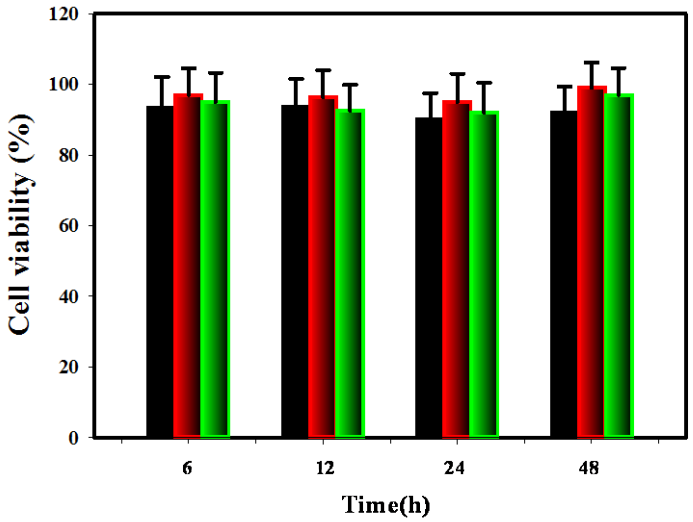

Fig. S7. Growth inhibition assay (MTT). LOVE-1 cells were incubated with unmodified AuNPs (1 nM), nanoflares (1 nM and 5 nM) for 6 h, 12 h, 24 h and 48 h. Blank bar stands for the unmodified Au NPs; red bar stands for the nanoflares (1 nM); green bar stands for higher concentration of the nanoflares (5 nM).

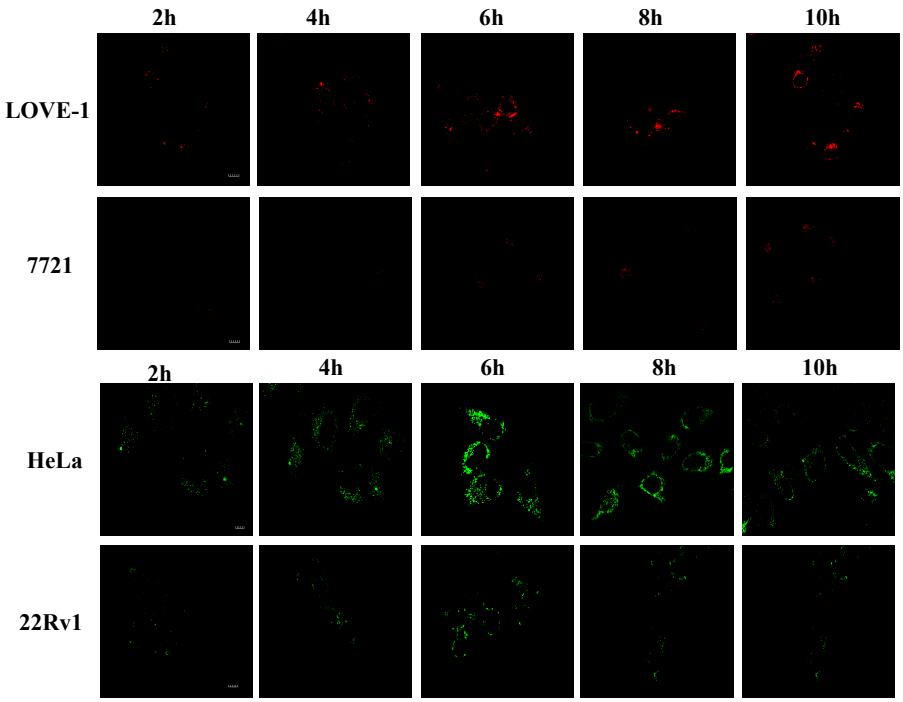

Fig. S8. Confocal images of four cells treated with nanoflares for 2,4,6,8 and 10 h at 37 ºC, 7721 and 22Rv1 cells were chosen as the control cells of Cy5 and FAM signal, respectively. The red fluorescence signals were recorded using Cy5 in the red channel with 633 nm excitation and the green fluorescence signals were recorded using FAM in the green channel with 488 nm excitation. Scale bar = 10 μm.

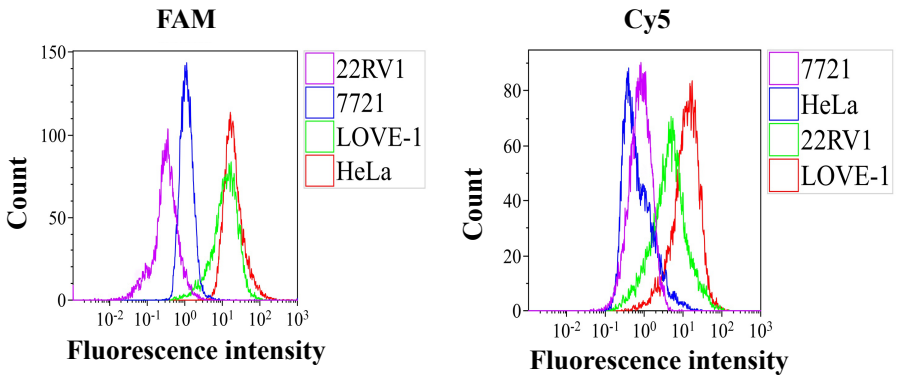

Fig. S9. Flow cytometry analysis of various cells type treated with nanoflares.

gene / Sample	miRNA-141	U6	ΔCt	ΔΔCt	$2^{-(\Delta\Delta Ct)}$
1	24.18793	12.85328	11.33464	0	1
2	29.4299	13.83117	15.59873	4.264091	0.052045
3	24.91745	14.24484	10.67261	-0.66203	1.582311
4	29.57694	14.77521	14.80173	3.467093	0.090428

Gene / Sample	miRNA-21	U6	ΔCt	ΔΔCt	$2^{-(\Delta\Delta Ct)}$
1	23.83093	12.85328	10.97765	0	1
2	24.08488	13.83117	10.25372	-0.72393	1.651675
3	22.84368	14.24484	8.598843	-2.37881	5.201058
4	23.26503	14.77521	8.489822	-2.48783	5.609318

Fig. S10. The expression analysis of miR-21 and miR-141 in four different cells respectively by qRT-PCR. Real-time fluorescence curves and relative expression level of miRNA-21and miRNA-141 in cells by qRT-PCR analysis.