Supporting Information:

Photo-Activated Nanoflares for mRNA Detection in Single Living Cells

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1. Supplementary Procedures

Quantification of DNA 1 Loaded on AuNPs.

The concentration of AuNPs in PC nanoflares was determined by UV-vis spectroscopy measurement. And the concentration of SNAs was determined by measuring the fluorescence of SNAs which displaced from the surface of AuNPs.[1] The displacement was achieved by adding MCH (final concentration 20 mM in 0.1 M PBS) into PC nanoflares solution, which was then incubated for 18 h with shaking at room temperature. Released DNA probes were then collected via centrifugation and the fluorescence was measured by a fluorescence spectrometer. The fluorescence intensity was converted to molar concentration of DNA by comparing to a standard linear calibration curve which was prepared using known concentrations of DNA 1 with identical buffer pH, ionic strength, and MCH concentration. All experiments were repeated three times.

Cell Viability Assay.

MTT assays were carried out to evaluate the potential cytotoxicity of the PC nanoflares and UV irradiation to the cells.[2] 2 nM PC nanoflares solution was incubated with MCF-7 cells for 8 h, 12 h, and 24 h, respectively. Subsequently, the cells were washed 3 times with PBS buffer, and fresh culture medium was added. Cells were irradiated by UV lamp (365 nm, 7000 μW/cm^2) for 5 min and incubated for another 12 h. Then 20 μL of MTT solution was added to each well and incubated for 4 h. After discarding the MTT solution, 200 μL of DMSO was added to each well to solubilize the formazan crystals, followed by shaking. Finally, the absorbance at 490 nm was measured using a TECAN microplate reader. The cells incubated with media only served as the control experiment.

2. Supporting information figures: Figure S1-S8

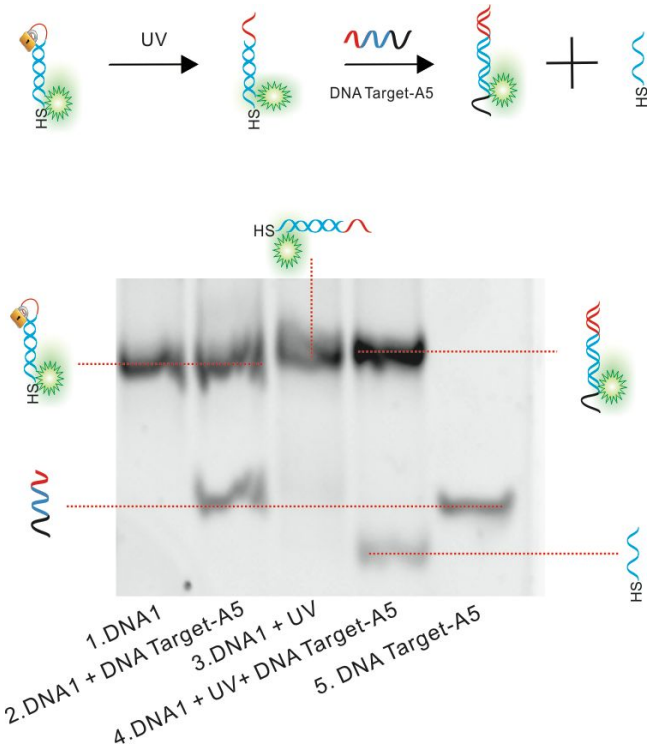

Figure S1. Native PAGE analysis shows that the UV irradiation could cleave PC linker in DNA 1 and activate toehold-mediated DNA strand displacement reaction. Lane 1: DNA 1, Lane 2: DNA 1 incubated with DNA Target-A5, lane 3: DNA 1 exposed to 365 nm UV light for 5 min, lane 4: after exposed to UV light, DNA 1 incubated with DNA Target-A5, lane 5: DNA Target-A5.

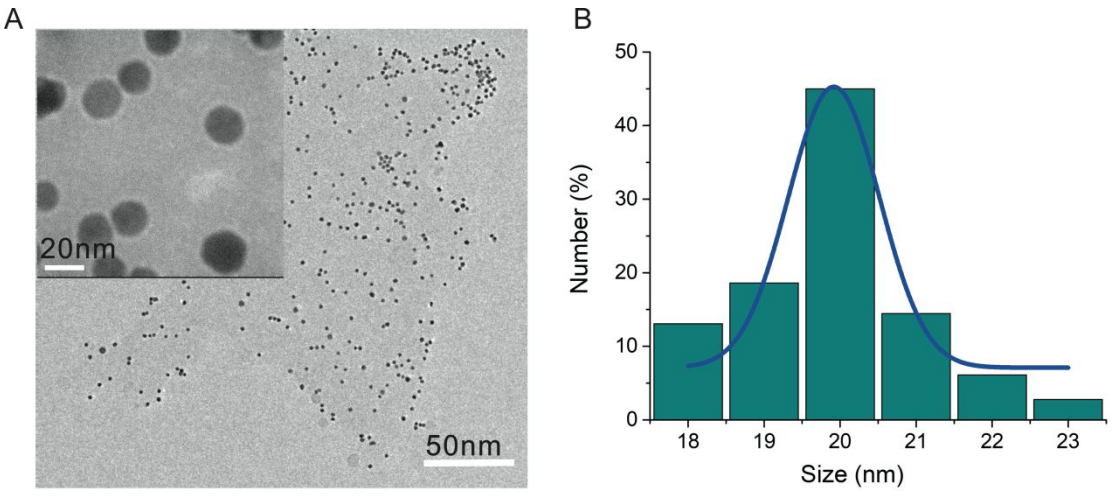

Figure S2. The characterization of AuNPs by TEM. (A) TEM image of AuNPs. The inset shows the zoom-in TEM images. (B) The sizes distribution of AuNPs was measured from more than 100 particles in TEM images.

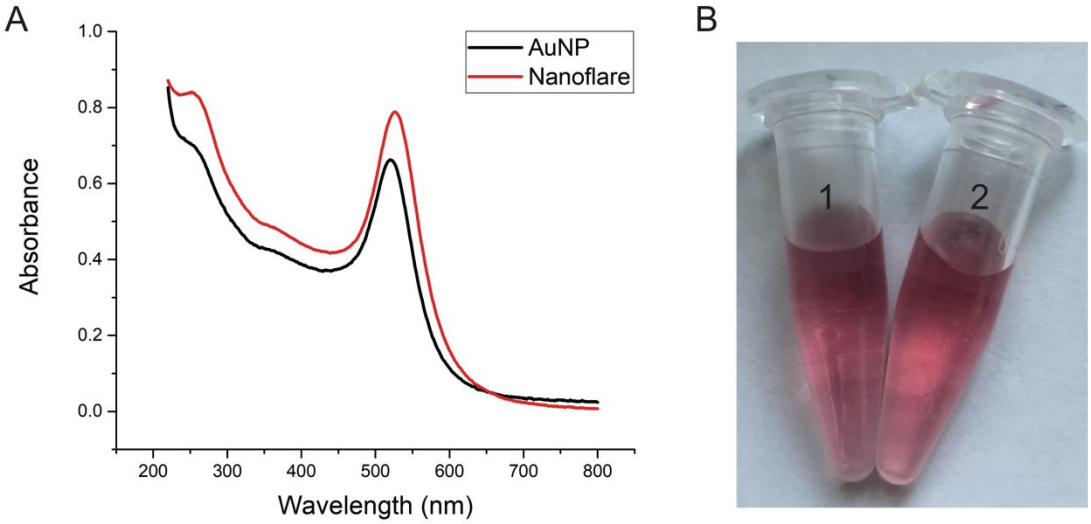

Figure S3. The characterizations of AuNPs and photo-activated nanoflares. (A) The UV-vis absorption of AuNP and PA nanoflares. The absorption peak of AuNPs is 524 nm. After functionalizing with DNA strands, a slight red (525 nm) shift occurs and the absorption peak of DNA appears at 260 nm. (B) Photographs of AuNP (1) and PA nanoflares (2).

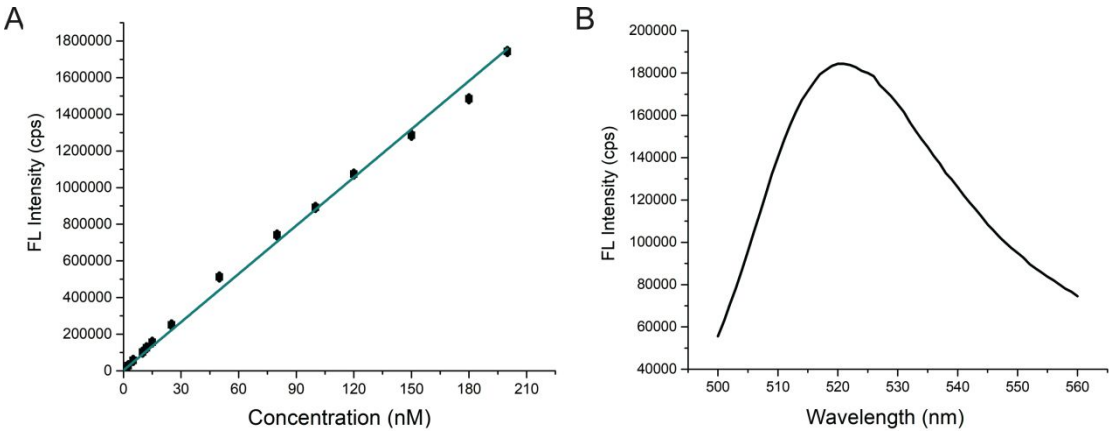

Figure S4. Evaluation of Amounts of hairpin DNA on each AuNP. (A) Standard linear calibration curve of fluorescence signal against the concentration of FAM labeled hairpin DNA. The excitation wavelength was 488 nm and the emission wavelength was

520 nm. The error bars represent the standard deviations. (B) Typical fluorescence spectrum of the supernatant after the treatment of the PA nanoflares with MCH to release the hairpin DNA.

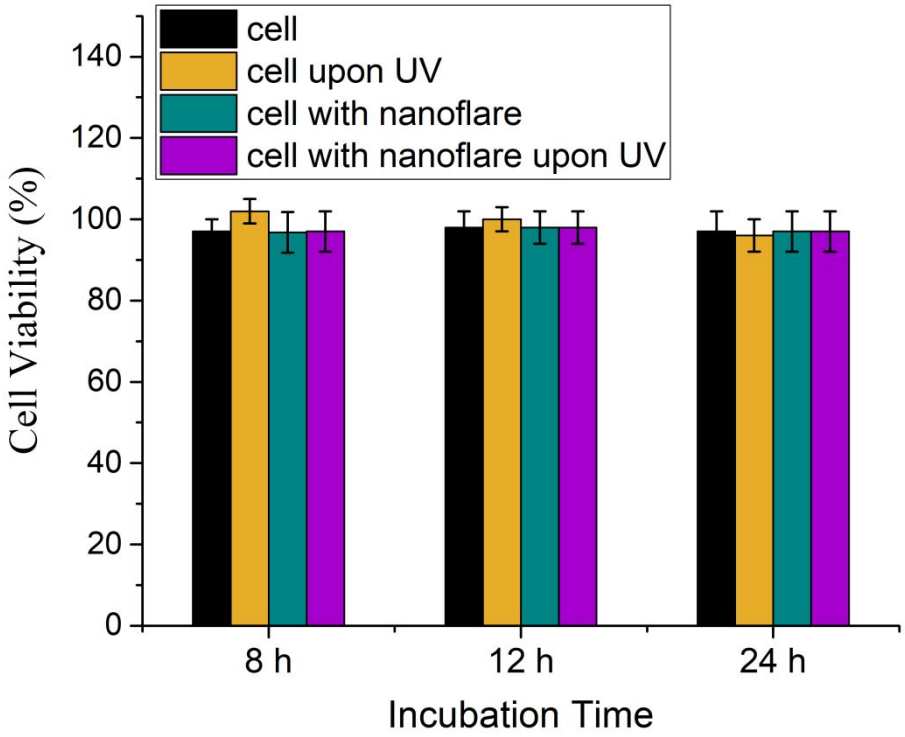

Figure S5. MTT assays of MCF-7 cells treated with the culture medium (black), UV irradiation (orange), PA nanoflares (dark cyan), and PA nanoflares with UV irradiation (dark cyan) with varying incubation time from 8 to 24 h, respectively.

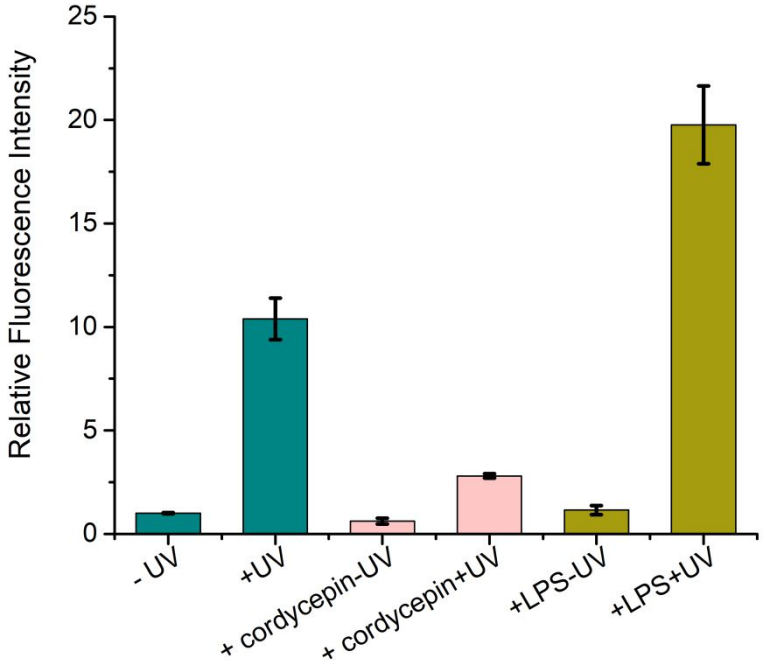

Figure S6. Relative fluorescence intensity under different conditions. Fluorescence intensity was calculated from Figure 3 by Carl Zeiss ZEN 2 (blue edition) software. The relative fluorescence value was calculated by dividing F by F_{-uv}, where F_{-uv} and F represent the averaged signal intensities of cells without UV irradiation in culture medium and cells treated with other conditions, respectively. Data were presented as the mean ± SEM.

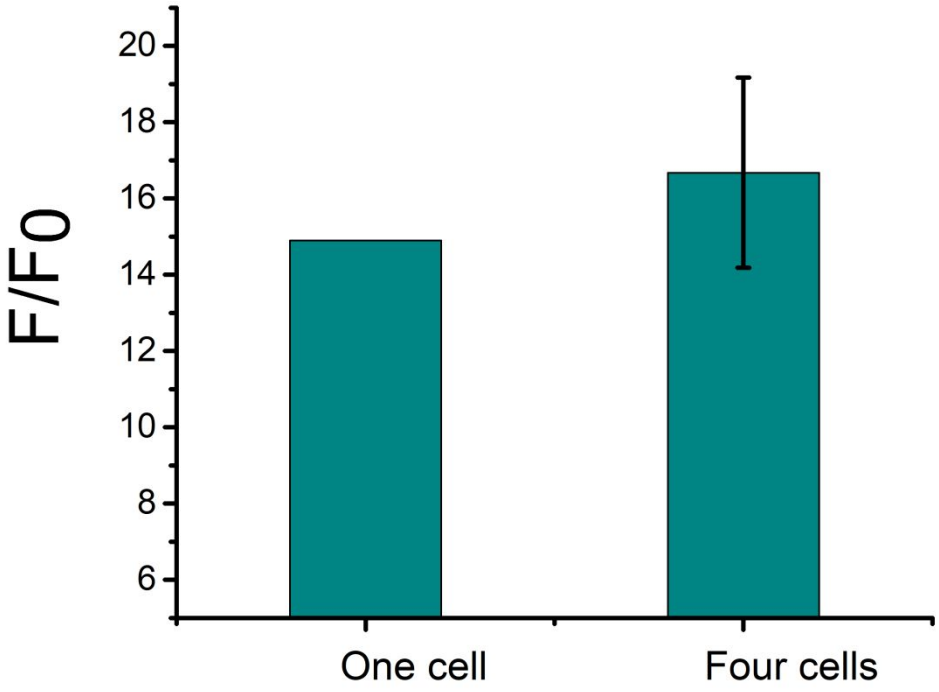

Figure S7. The ratio of fluorescence intensity at the single-cell level. Fluorescence intensity was calculated from Figure 4 by Carl Zeiss ZEN 2 (blue edition) software. The relative fluorescence value was calculated by dividing F by F_0, where F and F_0 represent the averaged signal intensities of cells after and before two-photo laser selective irradiation. Data were presented as the mean ± SEM.

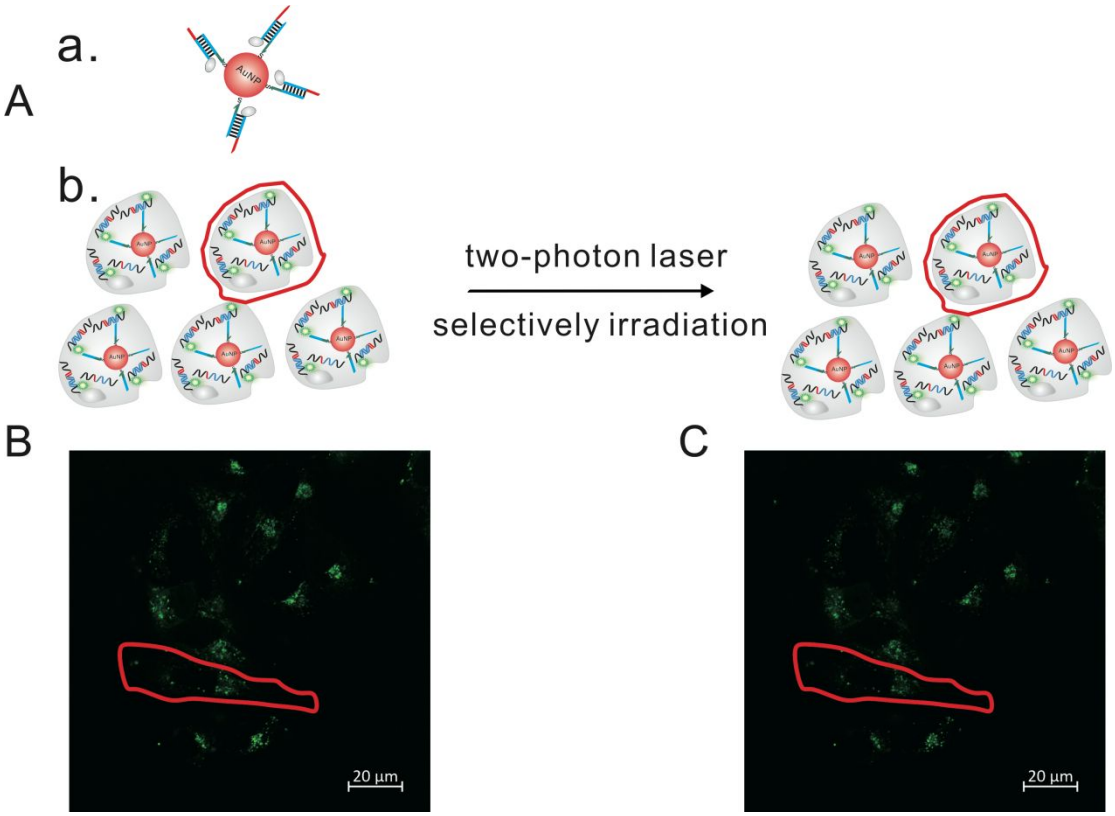

Figure S8. Conventional nanoflares for intracellular MnSOD mRNA detection before and after two-photo laser selective irradiation at the single-cell level. A. (a) Scheme of the conventional nanoflare. (b) Schematic illustration of the response of the conventional nanoflares to target MnSOD mRNA and the illumination by two-photo laser at 740 nm in a selective single living cell. Confocal images of MCF-7 cells excited at 488 nm before (B) and after (C) two-photo illumination at a selective single living cell (red contour).

REFERENCES

(1) Zhu, D.; Song, P.; Shen, J.; Su, S.; Chao, J.; Aldalbahi, A.; Zhou, Z.; Song, S.; Fan, C.; Zuo, X.; Tian, Y.; Wang, L.; Pei, H. *Anal. Chem.* **2016**, *88*, 4949-4954.

(2) Li, D.; Zhou, W.; Yuan, R.; Xiang, Y. *Anal. Chem.* **2017**, *89*, 9934-9940.