Electronic Supporting Information

A simple mitochondrion-targeting AIEgen for image-guided two-photon excited photodynamic therapy

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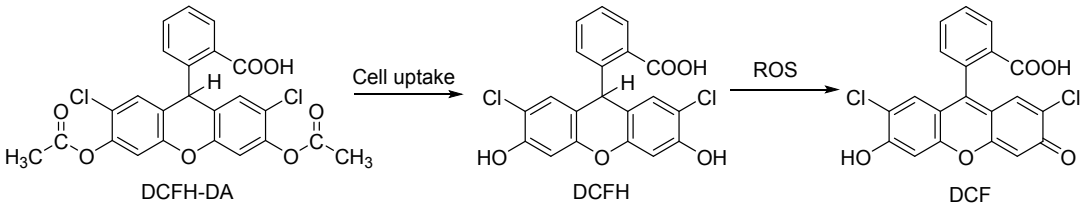

Scheme S1 ROS detection mechanism by DCFH-DA. Once the cell-permeable DCFH-DA was taken up by the cells, the acetyl groups were hydrolysed, giving DCFH. DCFH is sensitive to ROS and will be oxidized into a fluorescent compound DCF with excitation/emission of 488/525 nm.

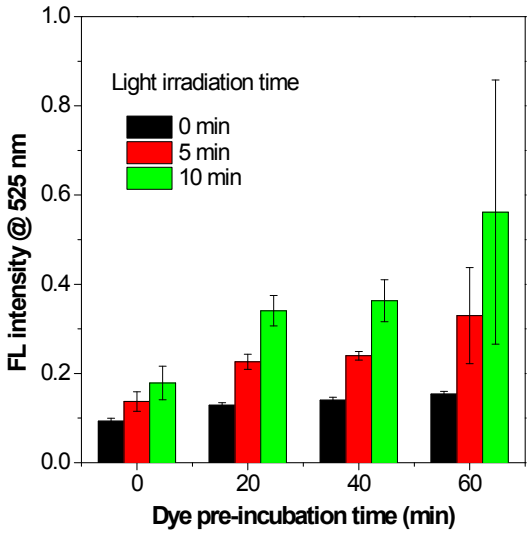

Fig. S1 Detection of ROS generation in cells by DCFH-DA. HeLa cells were incubated with 1 μM of IQ-TPA for different incubation time and then incubated with 10 μM of DCFH-DA for 30 min. After washed with PBS, the dye-stained cells were subjected to light irradiation for 0, 5 and 10 min. Fluorescence of the dye-stained cells was collected at 525±6 nm with excitation of 488 nm.

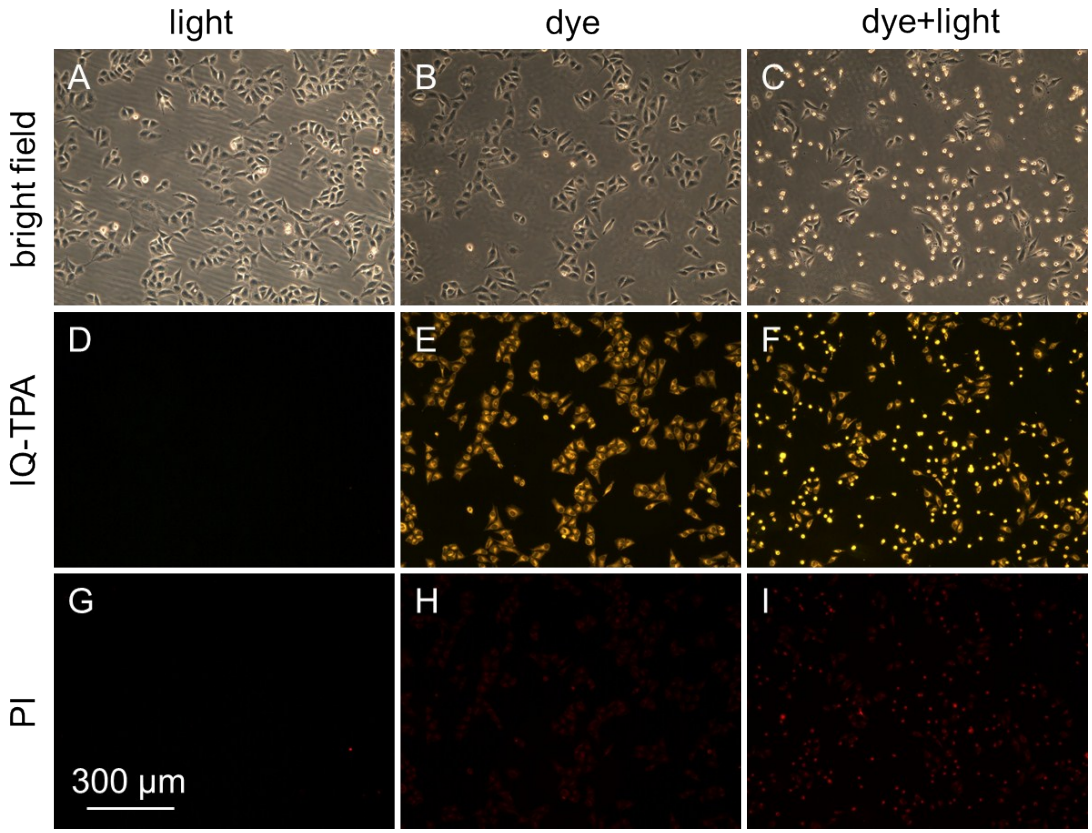

Fig. S2 HeLa cells were incubated with 1 μM IQ-TPA for 30 min with/without light irradiation for 15 min. After post-incubation for 3 h, cells were incubated with 1.5 μM PI for 15 min. (A–C) Bright field images and fluorescence images of (D–F) IQ-TPA and (G–I) PI. Excitation: 460–490 nm for IQ-TPA and 510–550 nm for PI.

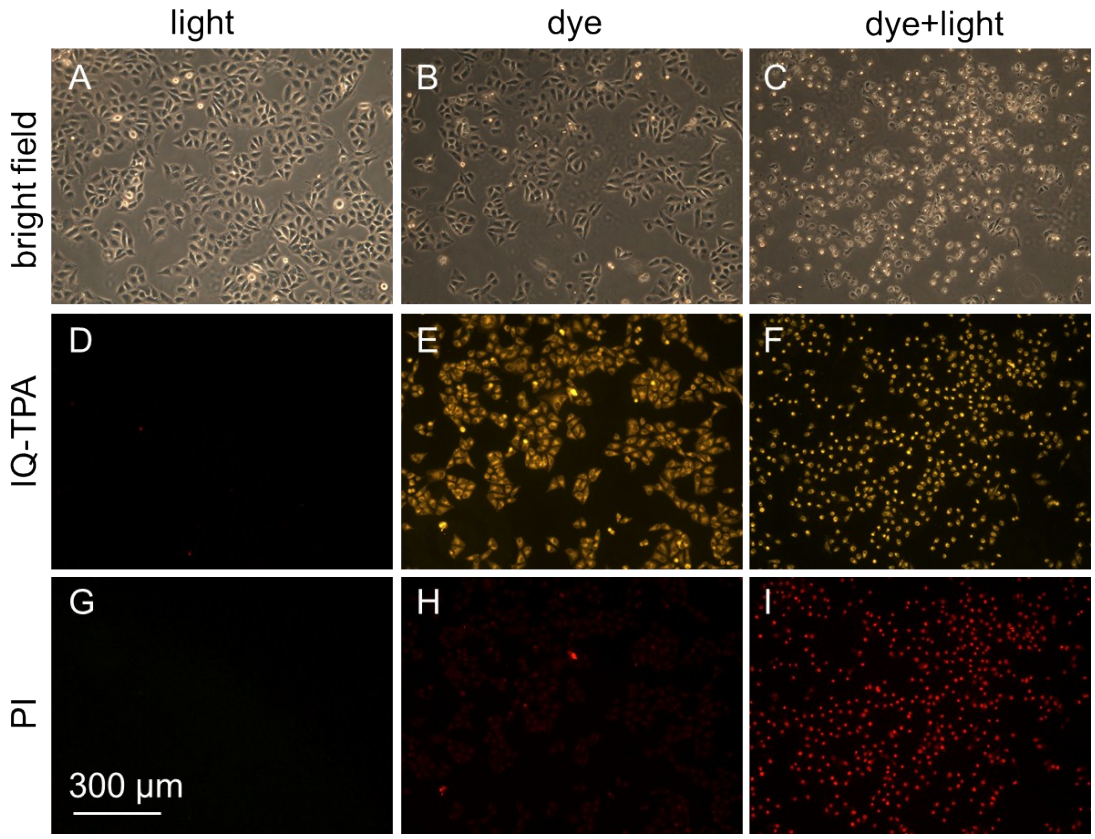

Fig. S3 HeLa cells were incubated with 1 μM IQ-TPA for 30 min with/without light irradiation for 15 min. After post-incubation for 18 h, cells were incubated with 1.5 μM PI for 15 min. (A–C) Bright field images and fluorescence images of (D–F) IQ-TPA and (G–I) PI. Excitation: 460–490 nm for IQ-TPA and 510–550 nm for PI.

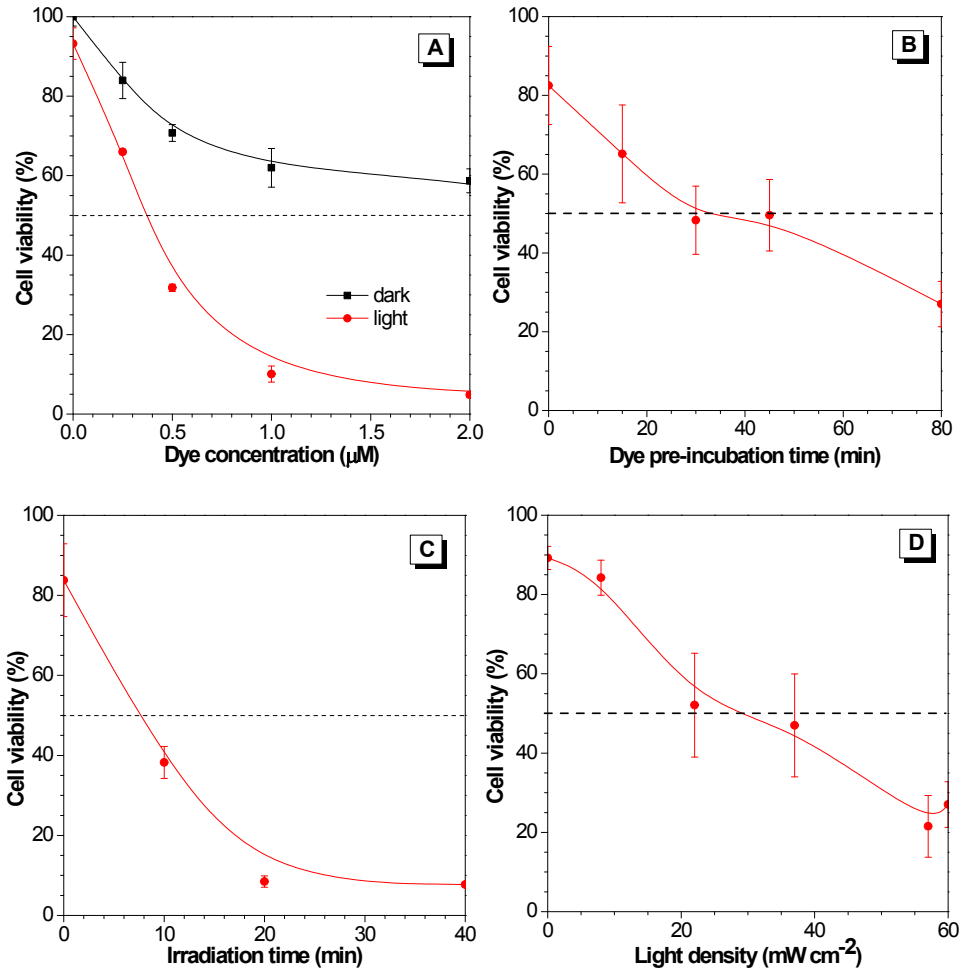

Fig. S4 Factors on photodynamic therapy: (A) Influence of dye concentration. Dye pre-incubation time = 30 min, light irradiation time = 15 min, light density = 60 mW/ cm^2. (B) Influence of dye pre-incubation time before light irradiation. Dye concentration = 1 μM, light irradiation time = 15 min, light density = 60 mW/ cm^2. (C) Influence of irradiation time. Dye concentration = 1 μM, dye pre-incubation time = 30 min, light density = 60 mW/ cm^2. (D) Influence of light density. Dye concentration = 1 μM, dye pre-incubation time = 90 min, light irradiation time = 15 min. HeLa cells were seeded in 24-well plate at the density of 30000/well and incubated for 24 h before experiments. After treatments, cells were further incubated for 24 h.

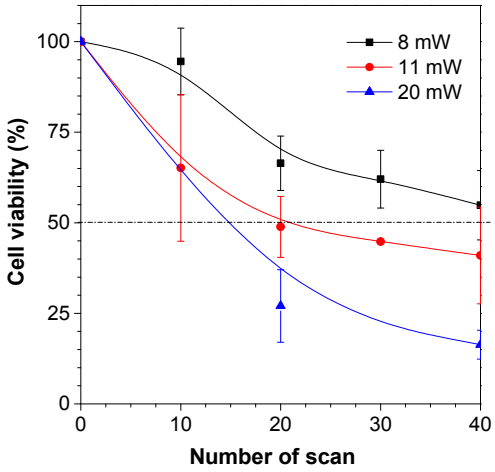

Fig. S5 In vitro cell viability after TP-PDT of IQ-TPA. Hela cells were incubated with IQ-TPA (1 μM, 30 min) and followed by different two-photon scans at the lase power of 8 mW, 11 mW or 20 mW. After 18-h post incubation, the cells were stained with fluorescein diacetate (FDA) and propidium iodode (PI) to assess the cell viability. The two-photon excitation condition was at 900 nm (fs Ti:sapphire laser) with a scan speed of 10.28 s/scan and a scanned area of 387.5 × 387.5 μm^2. Two or four parallel experiments were repeated.

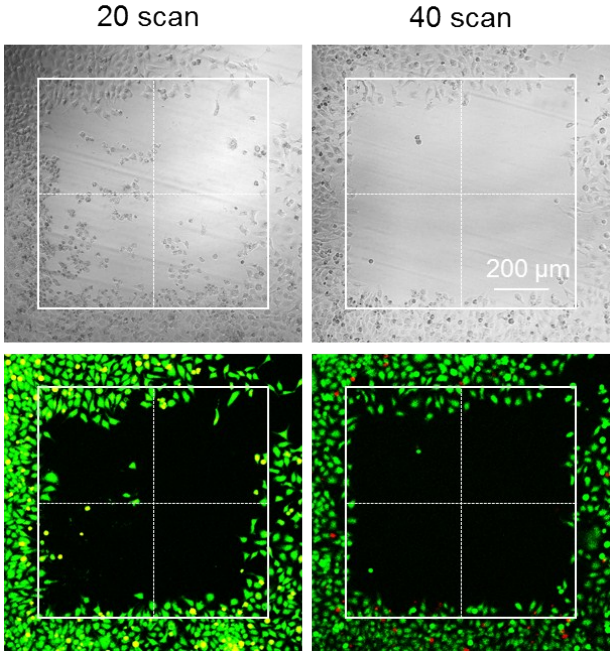

Fig. S6 Cell viabiltiy of HeLa cells treated with TP-PDT of IQ-TPA. Bright field and fluorescent overlay images of HeLa cells were shown. HeLa cells were incubated with IQ-TPA (1 μM, 30 min) and followed by different two-photon scans. After 18-h post-incubation, the cells were stained with FDA and PI to assess the cell viability. The two-photon excitation condition was at 900 nm (fs Ti:sapphire laser, 20 mW) with a scan speed of 10.28 s/scan. Four scanned areas of 387.5 × 387.5 μm^2 were shown by white squares. Excitation/emission: 488 /500–530 nm for FDA and 561 /600–650 nm for PI.

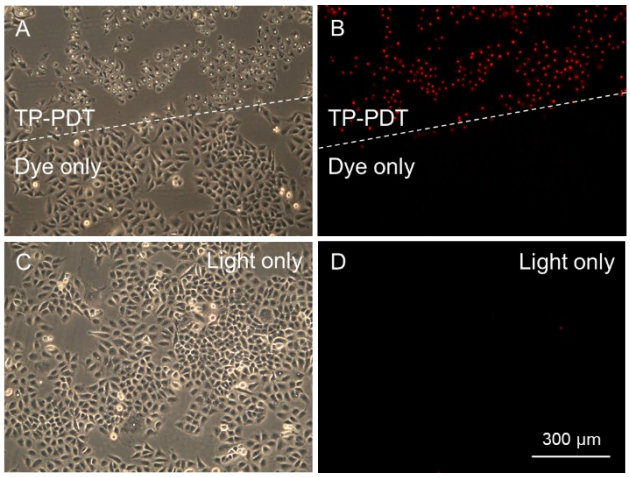

Fig. S7 (A and C) Bright field and (B and D) fluorescence images of treated HeLa cells stained with PI. (A and B) HeLa cells were incubated with 1 μM of IQ-TPA for 30 min and treated with/without two-photon scans. (C and D) HeLa cells were treated with two-photon scans alone. After post-incubation for 18 h, the cells were stained with 1.5 μM PI for 15 min. Two-photon excitation condition was at 900 nm (fs Ti:sapphire laser, 50 mW) with a scan area of 600 × 600 μm^2 and a scan speed of 8.19 s/scan. 110 scans were performed with a total time of 15 min. 4 scanned areas were neighboring to each other. Images were taken under fluorescence microscope with λ_{ex} = 510–550 nm for PI. The light dose was calculated to be 45000 J/cm^2 for a scan area of 600 × 600 μm^2 and estimated to be 7000 J/cm^2 for a scan area of 243 × 243 μm^2, which is comparable to the literature result of 7200 J/cm^2.[1] Similar, no signs of cytotoxicity to HeLa cells was found for the NIR laser irradiation alone.

References

1. B. Gu, W. Wu, G. Xu, G. Feng, F. Yin, P. H. J. Chong, J. Qu, K.-T. Yong and B. Liu, *Adv. Mater.*, 2017, **29**, 1701076.