Supporting Information

Biological Roles of Sulfur Dioxide and Sulfite in regulation of Mitochondria Viscosity

Weijie Zhang, [a] Yunxia Lv, [a] Hongjun Song, [b] Fangjun Huo, [b] Yongbin Zhang, [b] Caixia Yin, *[a]

a. Key Laboratory of Chemical Biology and Molecular Engineering of the Ministry of Education, Institute of Molecular Science, Shanxi University, Taiyuan 030006, China. E-mail: yincx@sxu.edu.cn
b. Research Institute of Applied Chemistry, Shanxi University, Taiyuan 030006, China

Table of contents:

1. General Information

1.1 Materials

All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. HeLa cells used in our experiment were purchased from Beyotime Institute of Biotechnology. Rhodamine 123 was purchased from Beyotime Institute of Biotechnology. Distilled water was used after passing through a water ultra-purification system. PBS buffer solution was obtained by mixing of 0.05mol/L Na_2HPO_4 water solution and 0.05mol/L KH_2P0_4 water solution with the volume ratio 4:1. Bisulfite and various analytes were purchased from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). Thiols-triggered SO_2 donor (T-G and T-R) was synthesized by our previous woks. All chemicals and solvents used were of analytical grade. All solution samples were made by dissolving their each solid in water or DMSO.

1.2 Instruments

TLC analysis was performed using precoated silica plates. Hitachi F-7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Shanghai Huamei Experiment Instrument Plants, China provided a PO-120 quartz cuvette (10 mm). ^{1}H NMR and ^{13}C NMR experiments were performed with a BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. ESI-MS was measured with a Thermo Scientific Q Exactive. The cells imaging experiments were measured by a Zeiss LSM-810 Airyscan confocal laser scanning microscope.

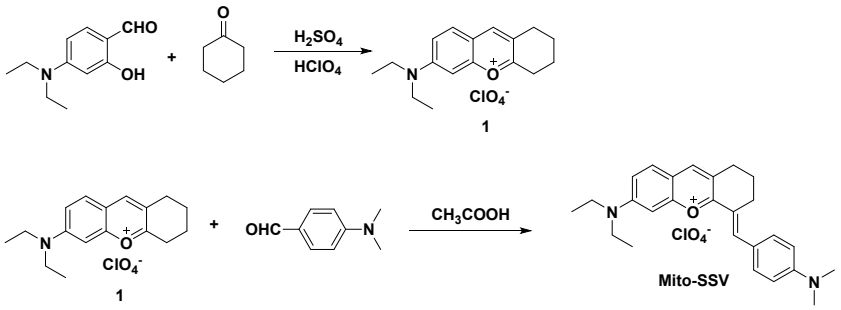
1.3 Bio-imaging

Cell Culture and Imaging. The cells were grown in Dulbecco's Modified Eagle's medium supplemented with 12% Fetal Bovine Serum and 1% antibiotics at 37 °C in humidified environment of 5% CO2. Cells were plated on 6-well plate and allowed to adhere for 24 h. Cells were incubated with

Mito-SSV at 37 ℃ for 20 min. Before the experiments, cells were washed with PBS for 3 times.

2. Experimental Section

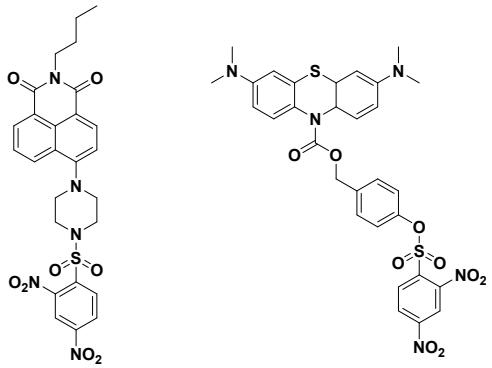
2.1 **Scheme S1**. Synthesis route of probe **Mito-SSV**.



2.2 Synthesis and characterization of **Mito-SSV.**

Compound **1** was prepared according to our previous reported works.[1] Then, compound **1** (0.711 g, 2 mmol) and 4-Dimethylaminobenzaldehyde (0.596 g, 4 mmol) was dissolved in 50 mL CH_3COOH. The mixture was heated at 110 °C for 4 h. After the reaction was completed, the solvent was removed to give the crude product. Then, dried and subjected to purification by flash chromatography (CH_2Cl_2 : CH_3OH; 20:1) to give probe **Mito-SSV** as a black solid (0.667 g, 34%). ^{1}H NMR (600 MHz, DMSO) δ 8.37 (s, 1H), 8.11 (s, 1H), 7.83 (d, J = 9.3 Hz, 1H), 7.66 (d, J = 8.7 Hz, 2H), 7.34 (d, J = 9.1 Hz, 1H), 7.23 (s, 1H), 6.87 (d, J = 8.7 Hz, 2H), 3.72 – 3.63 (m, 4H), 3.09 (s, 6H), 2.96 (s, 2H), 2.85 (s, 2H), 1.88 (d, J = 5.3 Hz, 2H), 1.25 (t, J = 6.9 Hz, 6H). ^{13}C NMR (151 MHz, DMSO) δ 164.78 (s), 158.43 (s), 155.19 (s), 152.24 (s), 147.06 (s), 140.05 (s), 134.72 (s), 131.80 (s), 123.41 (d, J = 9.9 Hz), 122.55 (s), 117.36 (s), 117.18 (s), 112.47 (s), 95.96 (s), 45.66 (s), 27.61 (s), 27.45 (s), 21.44 (s), 13.01 (s).

2.3 Chemical structure of T-G and T-R. Compound T-G and T-R was prepared according to our previous reported works.[2,3]



2.4 The mechanism of thiols-triggered SO2 release.

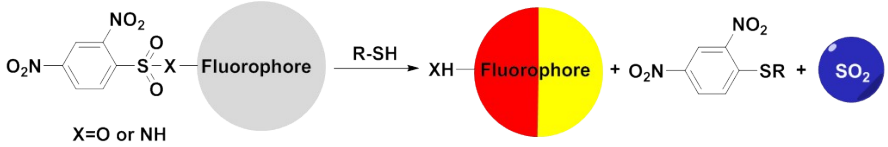
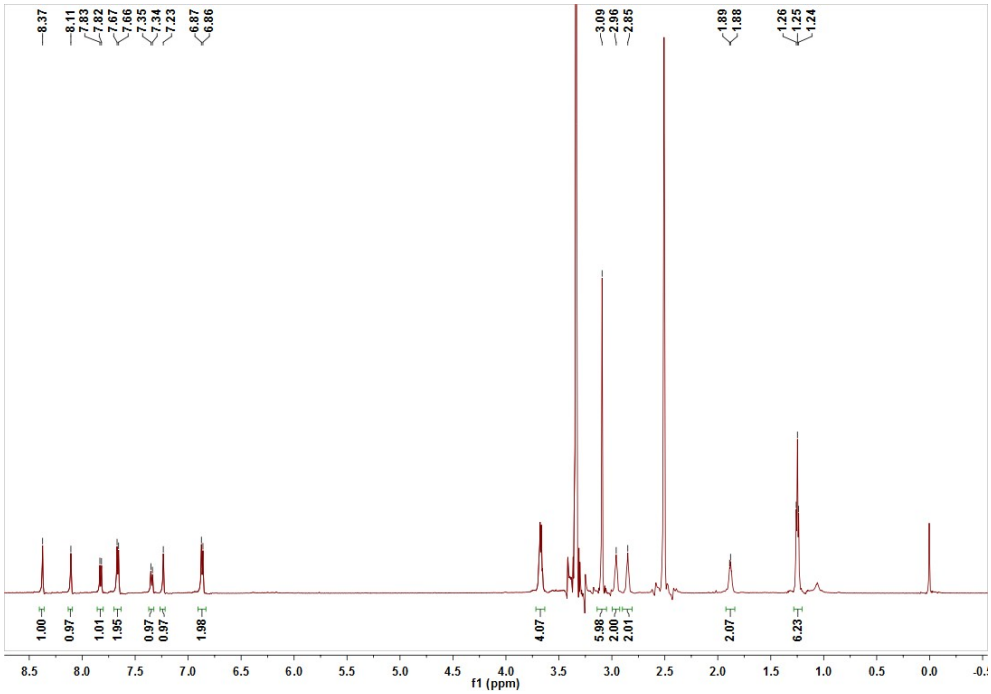
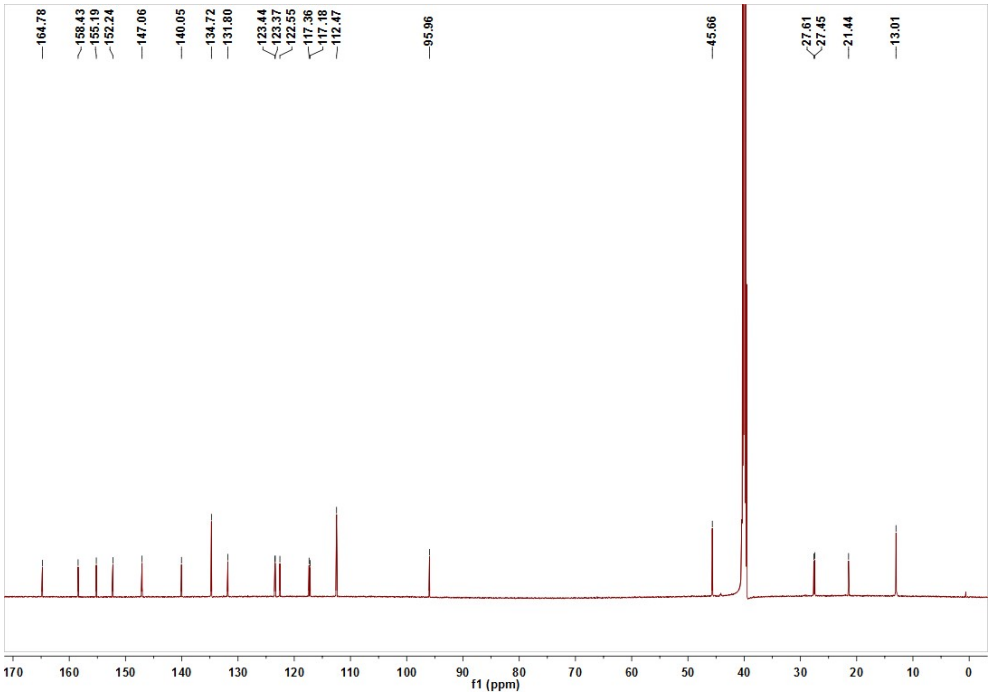


Figure S11: Structure characterization of **Mito-SSV**.



^{1}H-NMR spectrum of **Mito-SSV** in DMSO-d_6



^{13}C-NMR spectrum of **Mito-SSV** in DMSO-d_6

Figure S2: Fluorescence intensity changes at 740 nm of **Mito-SSV** in the presence of various analytes in the mixture of PBS and glycerin (v/v=1:1), (1) **Mito-SSV**, (2) Cys, (3) Hcy, (4) GSH, (5) GSH, (6) SO_4^{2-}, (7) SCN^-, (8) $S_2O_3^{2-}$, (9) AcO^-, (10) CO_3^{2-}, (11) H2O2, (12) NaClO.

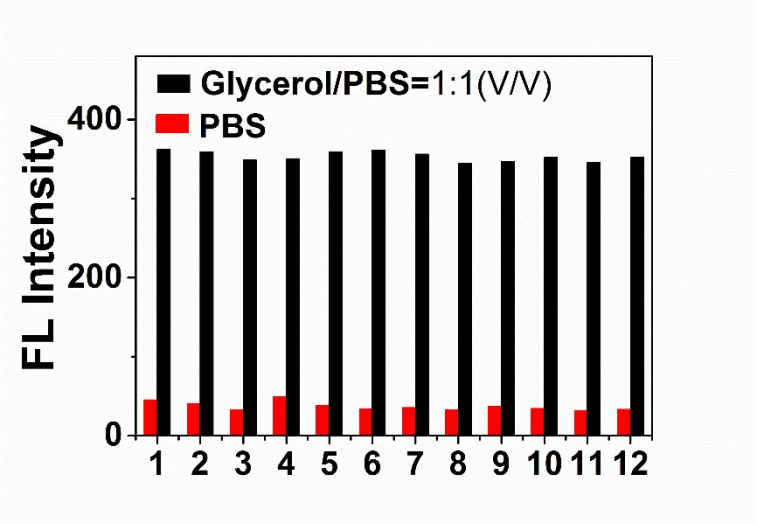


Figure S3: Fluorescence spectrum of **Mito-SSV** in different solvents.

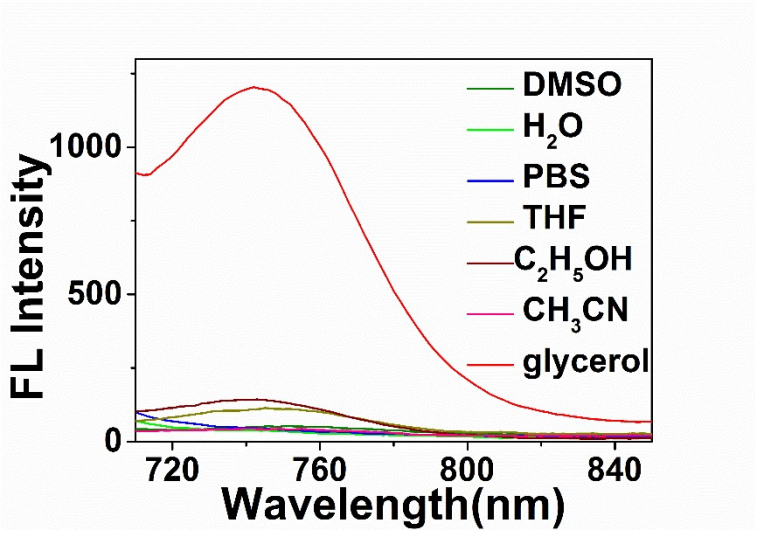


Figure S4: The cytotoxicity test of **Mito-SSV**.

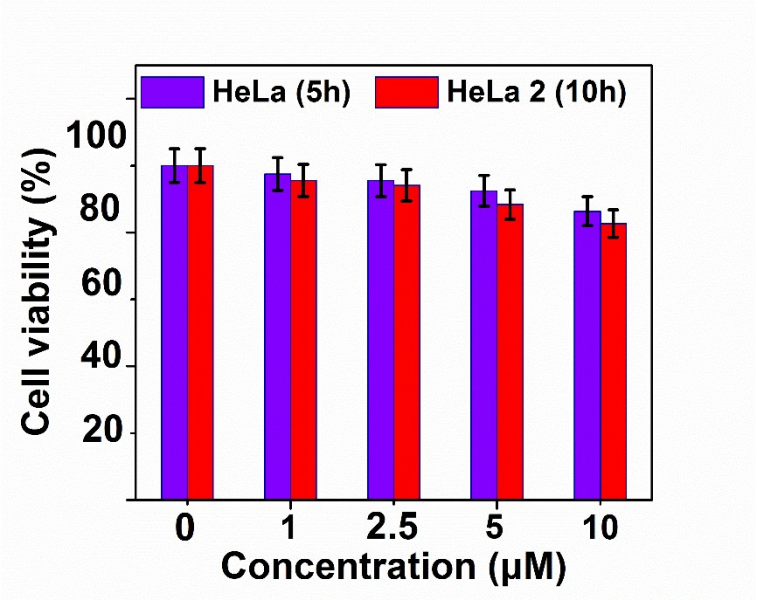


Figure S5: Mito-SSV and MitoTracker colocalization experiments in living HeLa cells.

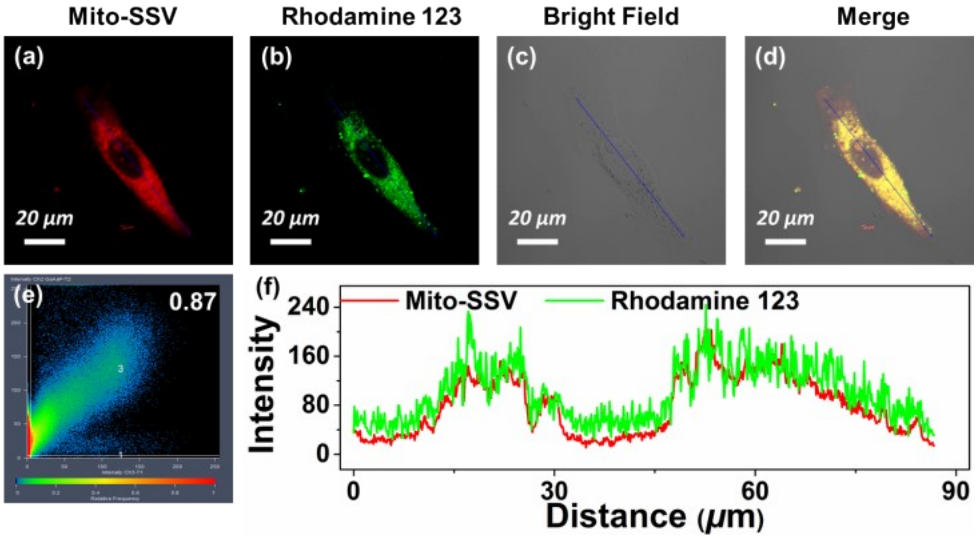


Figure S6: Time-dependent fluorescence images of probe **Mito-SSV** with H_2O_2 in HeLa cells.

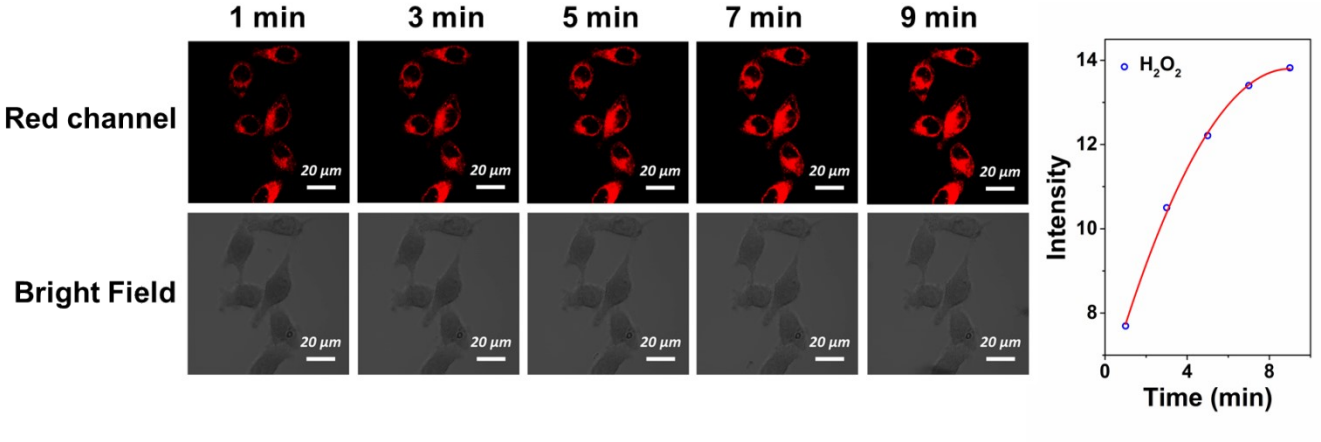


Figure S7: Time-dependent fluorescence images of probe **Mito-SSV** with HeLa cells.

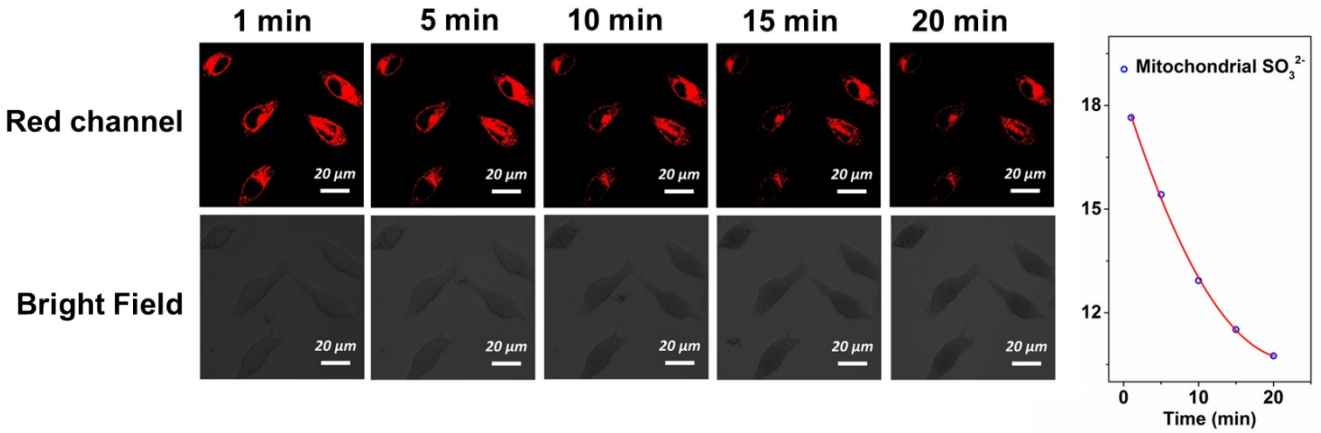


Figure S8: Time-dependent fluorescence images of probe **Mito-SSV** with Cys in HeLa cells.

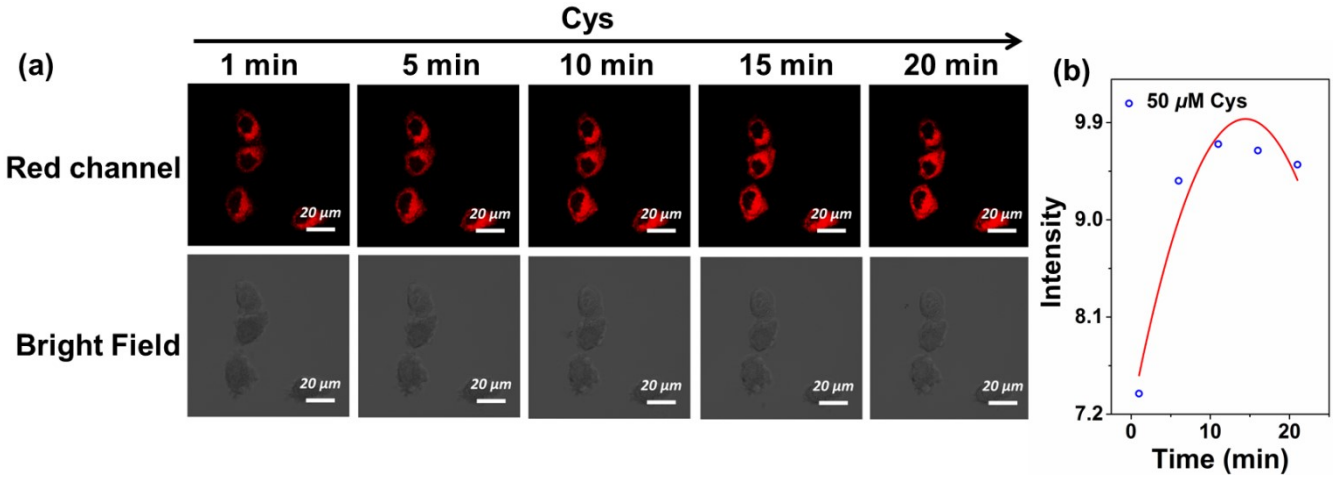
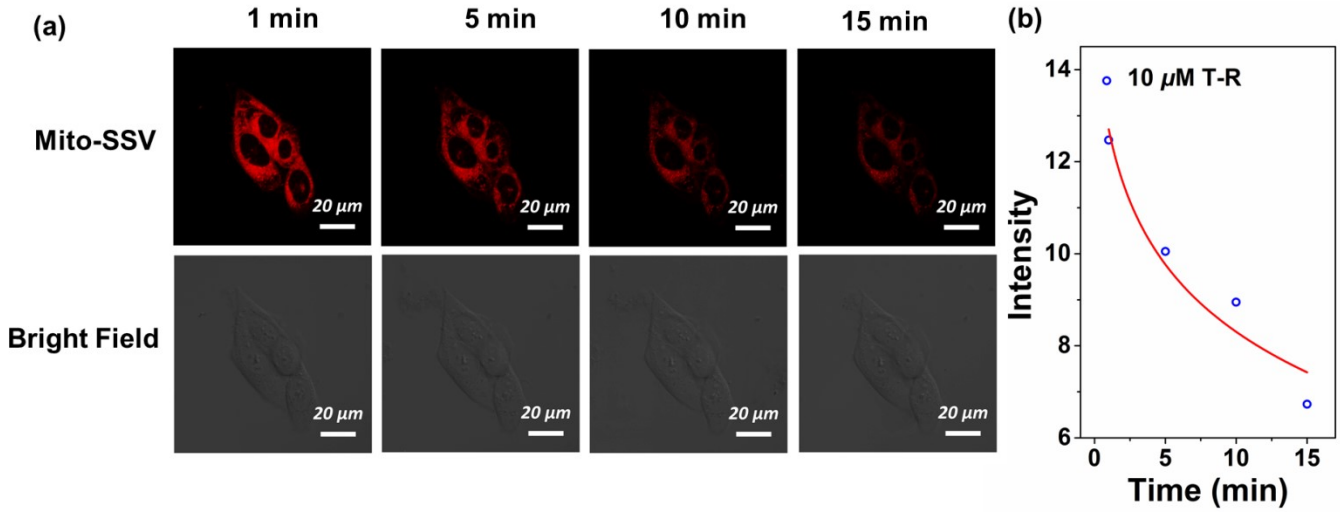


Figure S9: Fluorescent images of HeLa cells stained by **Mito-SSV** (10 μM) and then incubated with T-R (10 μM).



REFERENCES

1. Zhang, W. J.; Huo, F. J.; Zhang, Y. B.; Yin, C. X. *J. Mater. Chem. B,* **2019***, 7, 1945-1950*
2. W. J. Zhang, F. J. Huo, T. Liu, Y. Wen, C. X. Yin, *Dyes Pigments*, **2016**, *133***,** 248-254.
2. Wen, Y.; Long, Z. Q.; Bai, X. X.; Huo, F. J.; Yin, C. X. *Chem. Eng.J.* **2022**, *440*, 135978.