Electronic Supplementary Information

Phototherapeutic activity of polypyridyl ruthenium(II) complexes through synergistic action of nitric oxide and singlet oxygen

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Experimental section

1.1 Materials and apparatus

The reagents 5,6-diamino-1,10-phenanthroline, 2,2'-Bipyridine (bpy), 1,10-phenanthroline (phen), $RuCl_3$, LiCl, 4-Nitro-3-(trifluoromethyl)benzaldehyde, *N*,*N*-dimethylformamide (DMF), methanol, ethanol, ammonium hexafluorophosphate (NH_4PF_6) and DMSO-d_6 were purchased from Bide Pharmatech Co., Ltd. Cisplatin, dimethylsulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2'-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole trihydrochloride (Hoechst 33342), dichlorofluorescein diacetate (DCFH-DA), 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA) and rhodamine 123 (Rh123) were purchased from Sigma Aldrich. *N*-acetyl-*L*-cysteine (NAC) and catalase were obtained from Alfa Aesar. Mannitol, KI and superoxide dismutase (SOD) were purchased from J&K Scientific Ltd. The caspase-3/7 activity kit was purchased from Promega. The Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS) [6'-acetyloxy-4-amino-2',7'-difluoro-5-(methylamino)-3-oxospiro[2-benzofuran-1,9'-xanthene]-3'-yl] acetate (DAF-FM DA), tryptophan (Trp), carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO) and Annexin V-Fluorescein-5-isothiocyanate (Annexin V-FITC)/ Propidium Iodide (PI) apoptosis detection kit was received from Beyotime Biotech. Inc. ER-Tracker Red, MitoTracker Deep Red FM and LysoTracker Deep Red were purchased from Life Technologies. The antibodies were purchased from Cell Signaling Technology. The tested complexes were dissolved in 1% DMSO (v/v) for testing. All solvents used in the synthesis and cell-free experiments were of analytical grade.

^{1}H NMR spectrum was obtained in DMSO-d_6 using a 500 MHz Bruker Advance spectrometer. Absorption spectra were acquired using a Cary 60 spectrophotometer and a Hitachi F-7000 fluorescence spectrophotometer, respectively. Experiments with light irradiation were carried out with a blue LED array (λ_{ir} = 425 nm, 5 mW/cm^2). Flow cytometry experiments were performed on a Beckman Coulter flow cytometer and the data were analyzed using FlowJo software. Confocal microscopic images were acquired using a Leica TCS SP8 confocal microscope.

1.2 Cell lines and culture conditions

The human cervical cancer (HeLa), human lung carcinoma (A549) and human breast cancer

(MCF-7) cells were obtained from the Experimental and Animal Centre of Sun Yat-sen University. Cells were maintained in DMEM, which was supplemented with 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin, in a humidified incubator at 37 °C with 5% CO_2.

1.3 Synthesis of the Ru(II) complexes

Synthesis of 4-nitro-3-trifluoromethylaniline-1*H*-imidazo[4,5-*f*][1,10]phenanthroline (NFIP): 5,6-diamino-1,10-phenanthroline (210 mg, 1 mmol) and 4-nitro-3-trifluoromethylaniline (219 mg, 1 mmol) were mixed in 15 mL of anhydrous ethanol.[1] The mixture was refluxed at 80 °C celsius overnight. After the reaction, a substantial amount of yellow solid precipitates, which was then filtered and washed three times with anhydrous ethanol and anhydrous ether, respectively, before being dried for further use.

Synthesis of cis-$[Ru(bpy)_2(NFIP)](PF_6)_2$ (**Ru1**): Firstly, the *cis*-$Ru(bpy)_2Cl_2$ precursor was prepared. Bpy (3.44 g, 22 mmol), $RuCl_3$ (2.07 g, 10 mmol), and LiCl (2.12 g, 50 mmol) were mixed in 15 mL of DMF. The mixture was refluxed at 135 °C for 7 h. After reflux, the majority of the solvent was removed under reduced pressure, and 45 mL of H_2O was added to induce precipitation. The precipitate was filtered and washed extensively with H_2O until the filtrate was colorless, then dried for further use. Secondly, the synthesized *cis*-$Ru(bpy)_2Cl_2$ (97 mg, 0.2 mmol) and ligand NFIP (83 mg, 0.2 mmol) were dissolved in 12 mL of a methanol and H_2O mixture (3:1) under N_2 protection and refluxed for 8 h, resulting in a clear red solution. The solution was concentrated to 2 mL under reduced pressure; a saturated NH_4PF_6 solution was then added to precipitate a flocculent solid. This solid was filtered, washed multiple times with H_2O and ether, and dried under vacuum. The solid product obtained in the previous step was dissolved in a small amount of acetonitrile, followed by purification using column chromatography over neutral alumina with dichloromethane and acetonitrile as eluents. After drying, complex **Ru1** was obtained as a red solid, yield: 0.155 g, 70%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.98 (d, J = 8.2 Hz, 2H), 8.87 (s, 1H), 8.76 (d, J = 8.2 Hz, 5H), 8.39 (s, 4H), 8.31 (dd, J = 8.5, 5.3 Hz, 1H), 8.11 (dd, J = 13.9, 5.2 Hz, 4H), 7.84 (d, J = 4.7 Hz, 2H), 7.78 (td, J = 8.5, 5.3 Hz, 4H), 7.67 (dd, J = 7.9, 5.4 Hz, 2H). ESI-MS (CH_3OH): m/z calcd for $[M-2PF_6]^{2+}$, 411.5; found: 411.4; calcd for $[M-2PF_6-H]^+$, 822.1; found: 822.2. Elemental analysis calcd (%) for $C_{40}H_{26}N_9O_2F_{15}P_2Ru$: C, 43.18; H, 2.36; N, 11.33; found: C, 43.31; H, 2.40; N, 11.25.

Synthesis of $[Ru(phen)_2(NFIP)](PF_6)_2$ (**Ru2**): The first step involved the preparation of the *cis*-

$Ru(phen)_2Cl_2$ precursor. phen (3.96 g, 22 mmol), $RuCl_3$ (2.07 g, 10 mmol), and LiCl (2.12 g, 50 mmol) were mixed in 15 mL of DMF. The mixture was refluxed at 135 °C for 7 h. After reflux, the majority of the solvent was removed under reduced pressure, and 45 mL of H_2O was added to induce precipitation. The precipitate was filtered and washed extensively with H_2O until the filtrate was colorless, then dried for further use. Secondly, the synthesized *cis*-$Ru(phen)_2Cl_2$ (107 mg, 0.2 mmol) and ligand NFIP (83 mg, 0.2 mmol) were dissolved in 12 mL of a methanol and H_2O mixture (3:1) under N_2 protection and refluxed for 8 h, resulting in a clear red solution. This solid was filtered, washed multiple times with H_2O and ether, and dried under vacuum. The solid product obtained in the previous step was dissolved in a small amount of acetonitrile, followed by purification using column chromatography over neutral alumina with dichloromethane and acetonitrile as eluents. After drying, complex **Ru2** was obtained as a red solid, yield: 0.166 g 72%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.01 (d, J = 8.0 Hz, 2H), 8.86 (dd, J = 18.4, 8.2 Hz, 5H),8.78 (d, J = 8.5 Hz, 1H), 8.37 - 8.29 (m, 1H), 8.21 (t, J = 7.9 Hz, 2H), 8.10 (t, J = 7.9 Hz, 2H), 7.89 (s, 2H), 7.86 (d, J = 5.5 Hz, 2H), 7.80 (s, 2H), 7.59 (dd, J = 14.8, 6.4 Hz, 4H), 7.40 - 7.34 (m, 2H). ESI-MS (CH_3OH): m/z calcd for $[M-2PF_6]^{2+}$, 435.5; found: 435.4; calcd for $[M-2PF_6-H]^+$, 870.1; found: 870.3. Elemental analysis calcd (%) for $C_{44}H_{26}N_9O_2F_{15}P_2Ru$: C, 45.53; H, 2.26; N, 10.86; found: C, 45.64; H, 2.29; N, 10.95.

1.4 Measurement of $^1O_2$ quantum yield

Based on literature reference, the ability of complexes **Ru1** and **Ru2** to produce singlet oxygen ($^1O_2$) upon exposure to light in PBS was determined.[2] The probe ABDA acted as a $^1O_2$ scavenger. $[Ru(bpy)_3]Cl_2$ (**Ru-bpy**) was used as the standard (Φ_Δ = 0.18, in aerated PBS).[3] ABDA (100 μM) in PBS and the compounds being investigated were prepared in a cuvette under air equilibrium conditions. The absorbance of complexes **Ru1** and **Ru2** was adjusted to around 0.15 at 425 nm, followed by irradiation at room temperature with a 425 nm blue LED light (5 mW/cm^2). The absorbance of ABDA was measured every 80 s, and the $^1O_2$ quantum yield of the samples was calculated as per the method described in literature reference.

1.5 Photostability testing

The fluorescence spectral changes of the complexes **Ru1** and **Ru2** following illumination were analyzed. Complexes **Ru1** and **Ru2** were dissolved in CH_2Cl_2, CH_3OH or ethyl acetate to a final

concentration of 20 μM. These solutions were exposed to a 425 nm LED light (5 mW/cm^2) at 10 min intervals, and the alterations in their fluorescence spectra were documented. In addition, $[Ru(bpy)_3]Cl_2$ served as a reference to determine the fluorescence quantum yields of complexes **Ru1** and **Ru2** in CH_2Cl_2 solution.[4]

1.6 Distribution of the complexes in HeLa cells

Co-localization studies were conducted using the probe specific to the endoplasmic reticulum (ER-tracker Red), the probe specific to mitochondria (Mito-Tracker Deep Red FM), and the probe specific to lysosomes (LysoTracker Deep Red). HeLa cells were grown in 35 mm Corning dishes and cultured until they reached approximately 70% confluence. Then, the cells were exposed to 15 μM of complexes **Ru1** or **Ru2** in DMEM at 37 °C for 5 h. This was followed by a 30 min incubation with either ER-tracker Red (1.0 μM), Mito-Tracker Deep Red FM (150 nM), or LysoTracker Deep Red (150 nM). After two PBS washes, the cells were promptly examined using a Leica TCS SP8 confocal laser scanning microscope. The excitation and emission wavelengths were set as follows: **Ru1** and **Ru2**: Ex = 425 nm, Em = 475-550 nm; ER-Tracker Red: Ex = 552 nm, Em = 600-700 nm; MitoTracker Deep Red FM: Ex = 633 nm, Em = 670-720 nm; LysoTracker Deep Red: Ex = 633 nm, Em = 620-720 nm.

1.7 PDT activity

The MTT assay was employed to assess the PDT activity of complexes **Ru1** and **Ru2**. HeLa cells were collected via digestion, resuspended to form a single-cell suspension, adjusted to a concentration of 5 × 10^4 cells/mL, and plated into 96-well culture plates with 100 μL/well. Following a 24 h incubation at 37 °C, a 20 mM solution of the tested compound was prepared in DMSO and then diluted in the wells to concentrations ranging from 0 to 200 μM, while maintaining the final DMSO concentration in each well at 1% (v/v). Cells were treated with varying concentrations of the tested compound in the dark for 48 h, or exposed to 425 nm LED light (5 mW/cm^2) for 30 min, followed by incubation in the dark for 12 h and then an additional 36 h. Experiments were also performed under hypoxic conditions in both darkness and light. After incubation, 90 μL of DMEM and 10 μL of MTT solution (5 mg/mL) were added to each well. Following a 4 h of incubation, the culture medium was carefully removed, and 150 μL of DMSO was added to each well. Optical density (OD) at 570 nm was

measured using a microplate reader. Cell viability was calculated as the ratio of the average OD of treated wells to the average OD of control wells (cells exposed to light without the tested compound). Each treatment was repeated four times, and all experiments were conducted in triplicate.

1.8 ROS detection

ROS levels in HeLa cells were measured using confocal microscopy with the ROS probe DCFH-DA. The cells were seeded in 30 mm Corning cell culture dishes and treated with complexes **Ru1** or **Ru2** at different concentrations (0, 5.0, 10.0, and 20.0 μM) for 12 h. Afterward, cells were incubated with 10 μM DCFH-DA in the dark at 37°C for 20 min, followed by exposure to 425 nm LED light (5 mW/cm^2) for 30 min. Subsequently, the cells were rinsed twice with PBS and immediately examined using a Leica TCS SP8 confocal laser scanning microscope. DCF: Ex = 488 nm, Em = 500 - 540 nm.

1.9 NO detection

NO itself is highly unstable and readily oxidizes to form NO_2^- in cells or aqueous solutions. Therefore, the Griess assay was employed to detect the generation of NO by complexes **Ru1** and **Ru2** in the solvent.[5] Specifically, 50 μL of **Ru1** and **Ru2** or $NaNO_2$ (25, 50, and 100 μM) was added to each well of a 96-well plate, with three replicates per concentration. After 425 nm LED light (5 mW/cm^2) irradiation for 30 min, 50 μL of Griess Reagent I and 50 μL of Griess Reagent II were sequentially added to each well. The OD of each well was measured at 540 nm using a microplate reader. The NO concentration in the samples was calculated by comparison with $NaNO_2$.

Confocal microscopy was utilized to assess NO levels in HeLa cells using the NO probe DAF-FM DA. Cells were plated in 30 mm Corning cell culture dishes and exposed to complexes **Ru1** or **Ru2** at varying concentrations (0, 5.0, 10.0, and 20.0 μM) for 12 h. Afterward, the cells were incubated with 10 μM DAF-FM DA in the dark at 37°C for 20 min, followed by exposure under a 425 nm LED light (5 mW/cm^2) for 30 min. Subsequently, the cells were rinsed twice with PBS and immediately examined using a Leica TCS SP8 confocal laser scanning microscope. DAF: Ex = 495 nm, Em = 530 - 570 nm.

1.10 Intracellular ROS and NO scavenging test

The effect of ROS and NO on cell viability was assessed using ROS and NO scavengers. Cells were seeded in a 96-well plate and treated with ROS scavengers NAC (10 mM), tryptophan (20 μM),

NaN_3 (10 mM), SOD (1000 U/mL), mannitol (10 mM), Catalase (1000 U/mL), and KI (10 mM), and the NO scavenger Carboxy-PTIO (50 μM) in combination with 5 μM complexes **Ru1** or **Ru2** for 12 h. This was followed by incubation in the dark for 12 h or irradiation under a 425 nm LED light (5 mW/cm^2) for 30 min and then further incubation for 12 h. After incubation, cell viability was measured using the MTT assay.

1.11 Mitochondrial membrane potential (MMP) detection

Confocal microscopy was assessed the impact of complexes **Ru1** and **Ru2** on the MMP in HeLa cells, employing Rh123. HeLa cells were plated in 30 mm Corning cell culture dishes and exposed to complexes **Ru1** or **Ru2** at various concentrations (0, 5.0, 10.0, and 20.0 μM) for 12 h. Following either incubation in the dark or exposure to 425 nm LED light (5 mW/cm^2) for 30 min, the cells were washed twice with PBS. Subsequently, the cells were stained with Rh123 (5 μg/mL) at 37°C for 15 min. Subsequently, the cells were washed twice with PBS and promptly examined using a confocal microscope. Rh123: Ex = 488 nm, Em = 515 - 545 nm.

1.12 Hoechst 33342 staining

Confocal microscopy was employed to evaluate the effects of complexes **Ru1** and **Ru2** on the nuclear morphology of HeLa cells using Hoechst 33342. Cells were seeded in 30 mm Corning cell culture dishes and treated with **Ru1** or **Ru2** complexes at different concentrations (0, 5.0, 10.0, and 20.0 μM) for 12 h. This was followed by either incubation in the dark for another 12 h or exposure to 425 nm LED light (5 mW/cm^2) for 30 min, and then an additional 12 h incubation. After fixation with 4% paraformaldehyde for 10 min at room temperature, the cells were gently washed twice with PBS to ensure suspended cells were not removed. They were subsequently stained with Hoechst 33342 (5 μg/mL) at 37 °C for 15 min. Finally, the cells were carefully washed twice with PBS before being examined with a confocal microscope. Hoechst 33342: Ex = 405 nm, Em = 440 - 480 nm.

1.13 Annexin V-FITC/PI double staining for cell apoptosis detection

Flow cytometry: HeLa cells in 6-well plates were treated with various concentrations of complexes **Ru1** or **Ru2** (0, 5.0, 10.0, and 20.0 μM) for 12 h. This was followed by incubation in the dark for 12 h or exposure to 425 nm LED light (5 mW/cm^2) for 30 min, then further incubation for 12 h. After this period, cells were harvested with trypsin, resuspended in a mixture containing 5 μL

Annexin V-FITC, 10 μL PI, and 200 μL Annexin V binding buffer, and stained for 15 min in the dark at room temperature. Stained cells were immediately analyzed using a Beckman Coulter flow cytometer. The data were analyzed using FlowJo software.

Confocal microscopy: Cells were plated in 30 mm Corning cell culture dishes and treated with **Ru1** or **Ru2** at varying concentrations (0, 5.0, 10.0, and 20.0 μM) for 12 h. This treatment was followed by either a 12 h incubation in the dark or exposure to 425 nm LED light (5 mW/cm²) for 30 min, with an additional 12 h incubation thereafter. The cells were washed twice with PBS to prevent the loss of suspended cells and subsequently stained with 5 μL Annexin V-FITC, 10 μL PI, and 200 μL Annexin V binding buffer at room temperature in the dark for 15 min. After staining, the cells were again washed twice with PBS and immediately examined using a confocal microscope. Annexin V-FITC: Ex = 488 nm, Em = 520 - 560 nm, PI: Ex = 535 nm, Em = 570 - 660 nm.

1.14 Caspase-3/7 activity assay

The activity of Caspase-3/7 in HeLa cells was evaluated using the Promega Caspase-Glo® Assay Kit, following the protocol outlined in previous studies.[6] Cells were plated in a 96-well plate and treated with various concentrations of complexes **Ru1** or **Ru2** (0, 5.0, 10.0, and 20.0 μM) for 12 h, This treatment was followed by either a 12 h incubation in the dark or irradiation with a 425 nm LED light (5 mW/cm²) for 30 min, and then an additional 12 h incubation. After incubation period, 100 μL of DMEM and 100 μL of Caspase-3/7 reagent were added to each well and incubated at room temperature for 1 h. Cisplatin (20 μg/mL) was used as the positive control. Fluorescence was measured using a microplate reader.

2 Supplementary table

Table S1 The IC_{50} of **Ru1** and **Ru2** under darkness, or light irradiation (425 nm) for A549 or MCF-7 cells

	IC_{50} (μM), A549		IC_{50} (μM), MCF-7	
Complex	Dark[a] (light)[b]	PI[c]	Dark[a] (light)[b]	PI[c]
Ru1	>200 (3.55 ± 0.77)	>56.34	>200 (3.38 ± 0.54)	>59.17
Ru2	>200 (3.31 ± 0.86)	>60.42	>200 (3.98 ± 0.17)	>50.25

[a] Cells were treated with the tested complexes for 48 h. [b] Cells were treated with the tested complexes for 12 h before irradiation. [c] PI = IC_{50}(dark)/IC_{50}(light).

3 Supplementary figures

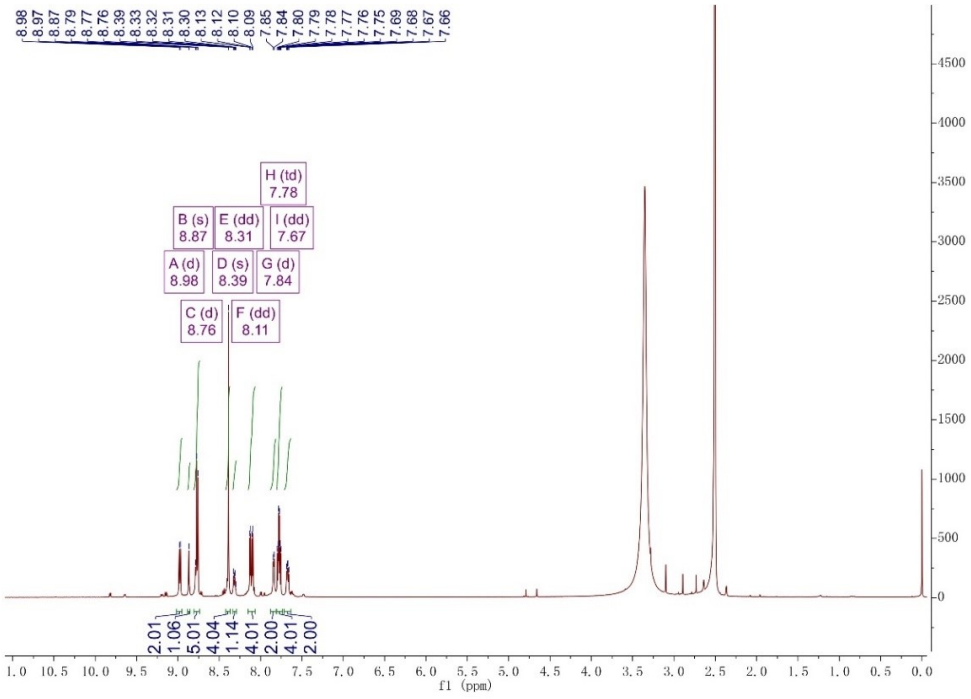


Fig. S1 ^{1}H NMR spectrum of **Ru1** in DMSO-d_6 at 298 K.

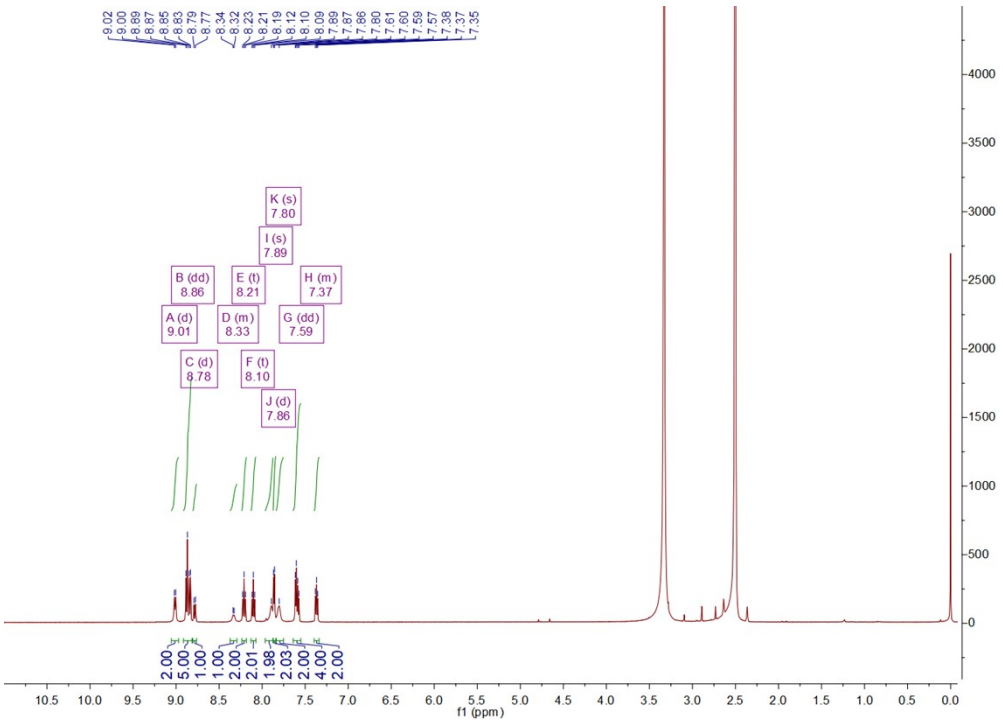


Fig. S2 ^{1}H NMR spectrum of **Ru2** in DMSO-d_6 at 298 K.

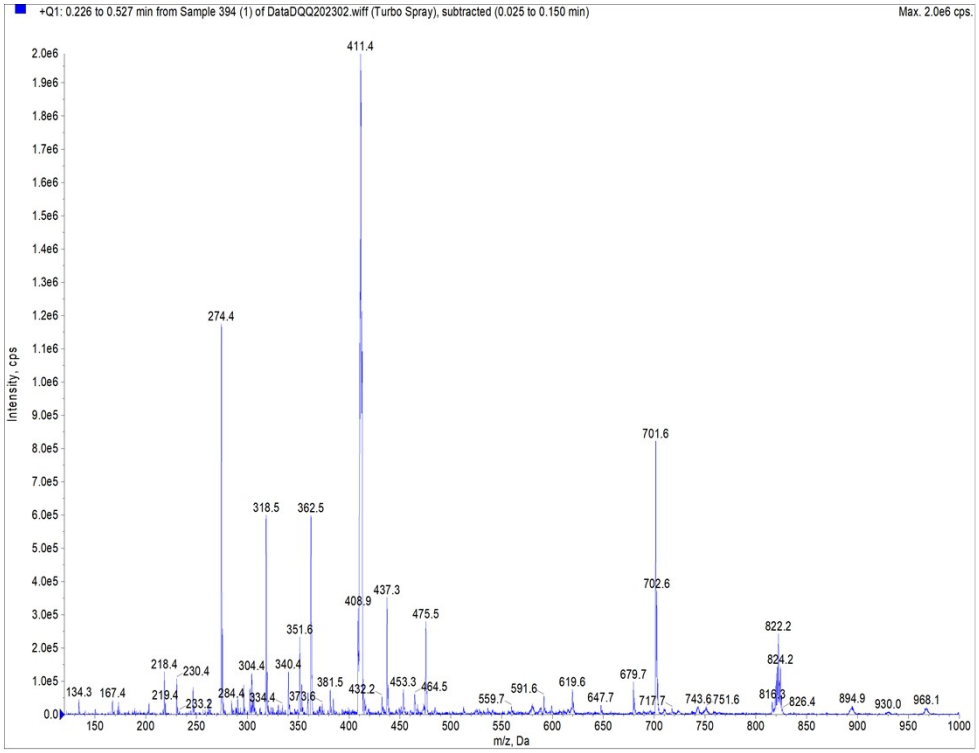


Fig. S3 Mass spectrometry of **Ru1**.

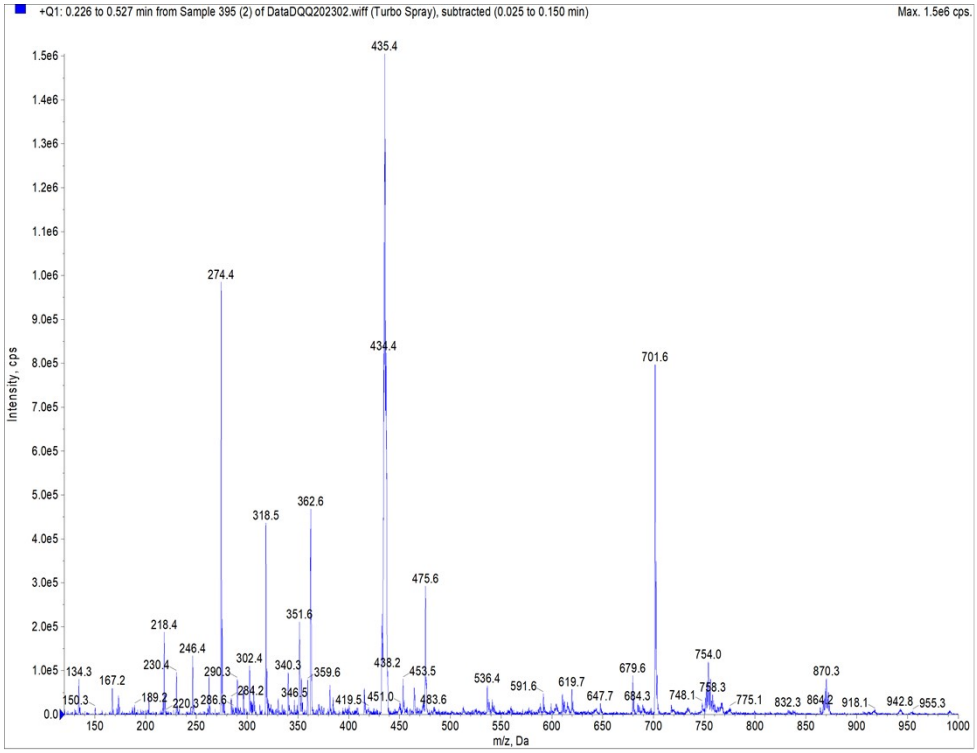
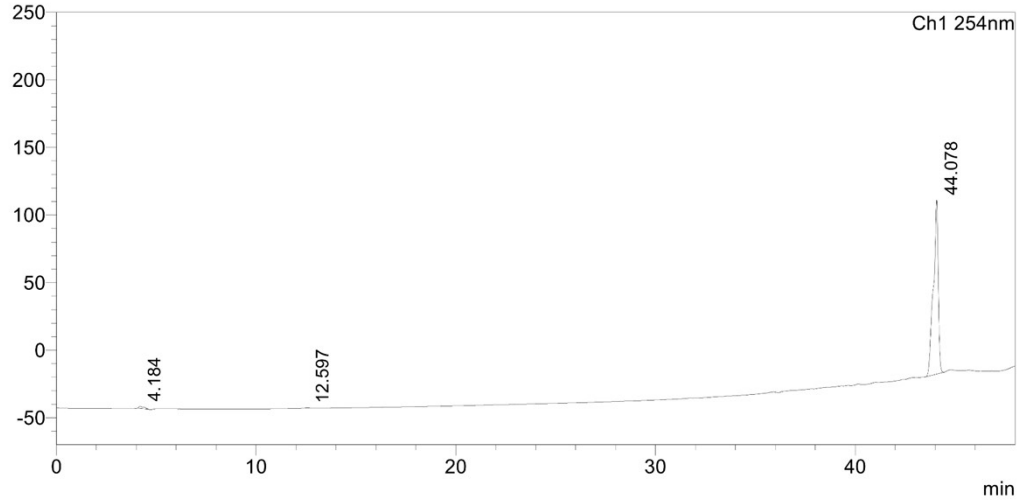


Fig. S4 Mass spectrometry of **Ru2**.

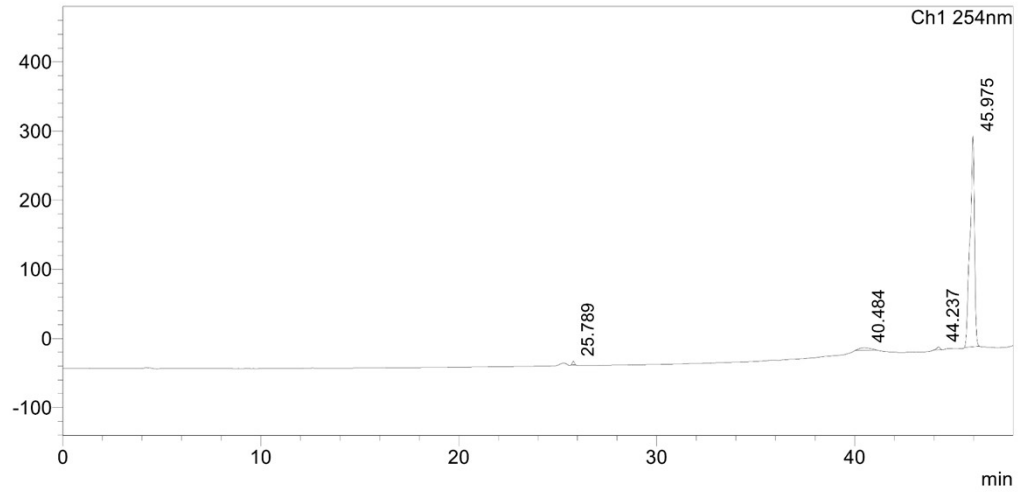


<Peak Table>

Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	4.184	28118	1503	1.269		M	
2	12.597	618	86	0.028		M	
3	44.078	2187474	128843	98.703		M	
Total		2216209	130431				

Fig. S5 Purity analysis result of **Ru1** by HPLC.



<Peak Table>
Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	25.789	38198	5308	0.756		M	
2	40.484	138347	3470	2.739		M	
3	44.237	50427	4245	0.998		M	
4	45.975	4824538	304183	95.507		M	
Total		5051510	317206				

Fig. S6 Purity analysis result of **Ru2** by HPLC.

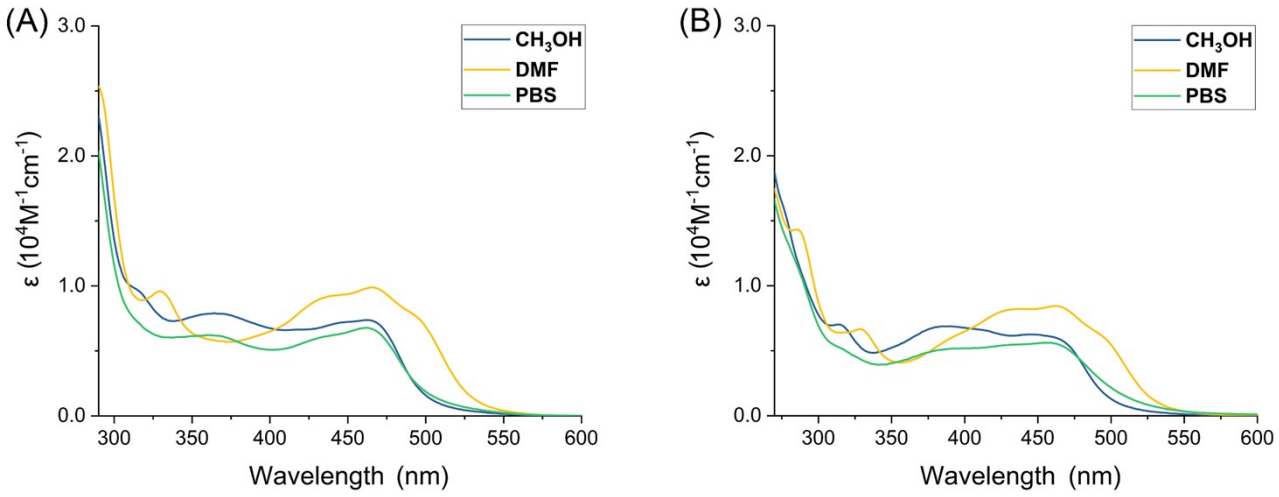


Fig. S7 UV/Vis absorption spectra of (A) **Ru1** and (B) **Ru2** (10 μM) measured in CH_3OH, DMF and PBS.

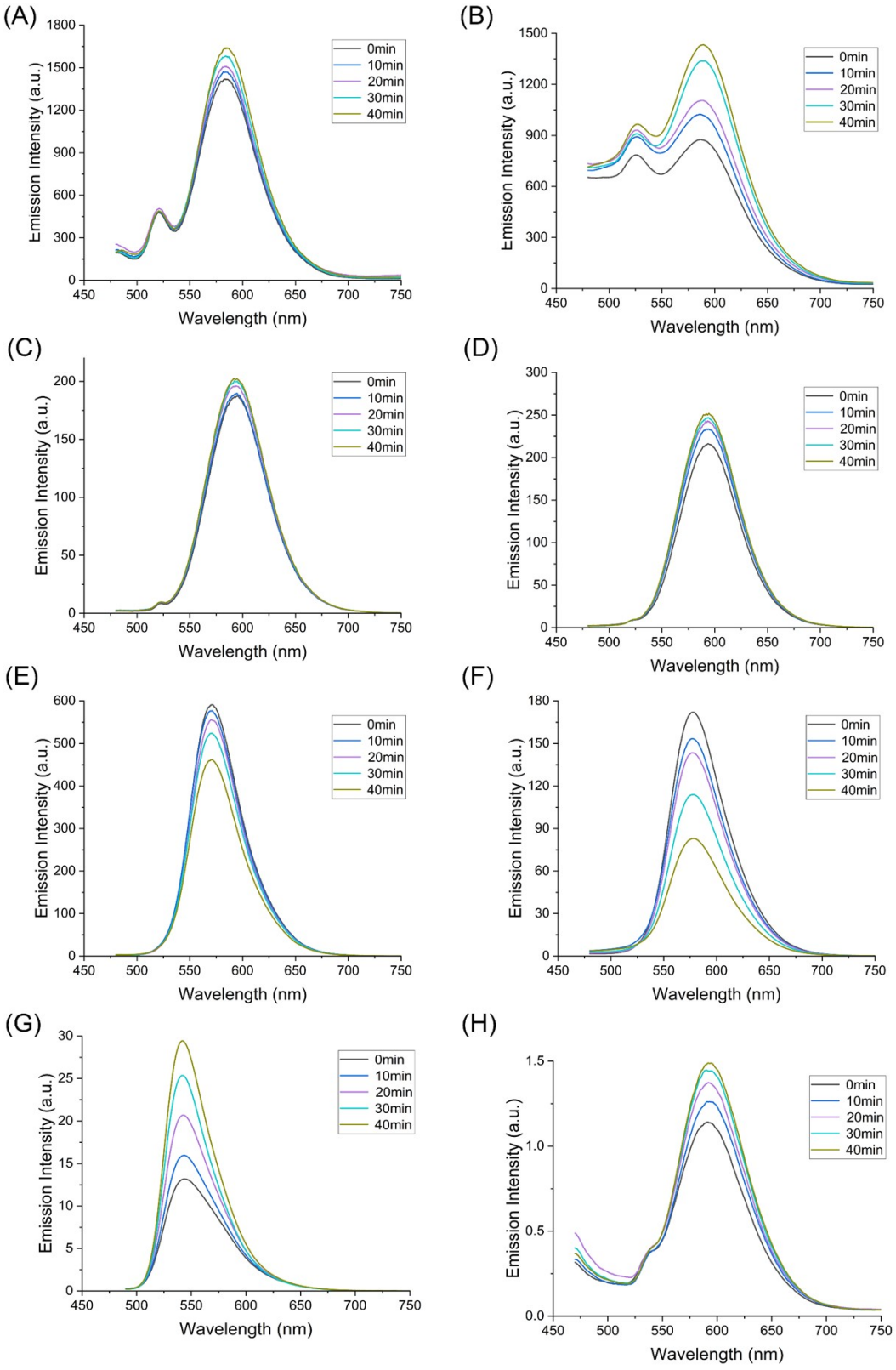


Fig. S8 Emission spectra of (A,C,E) **Ru1** and (B,D,F) **Ru2** (20 μM) measured in (A,B) CH_3OH, (C,D) ethyl acetate, (E,F) CH_2Cl_2 and (G,H) PBS over time at 298 K.

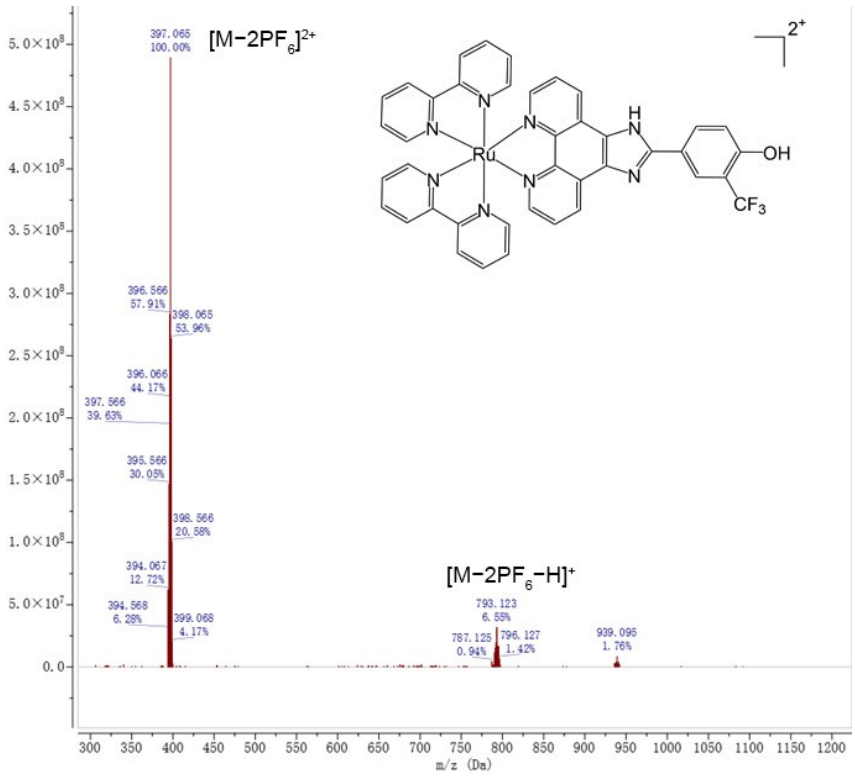


Fig. S9 High-resolution mass spectrometry of the photodegraded product of **Ru1** (5 μM). Irradiation time: 1 h.

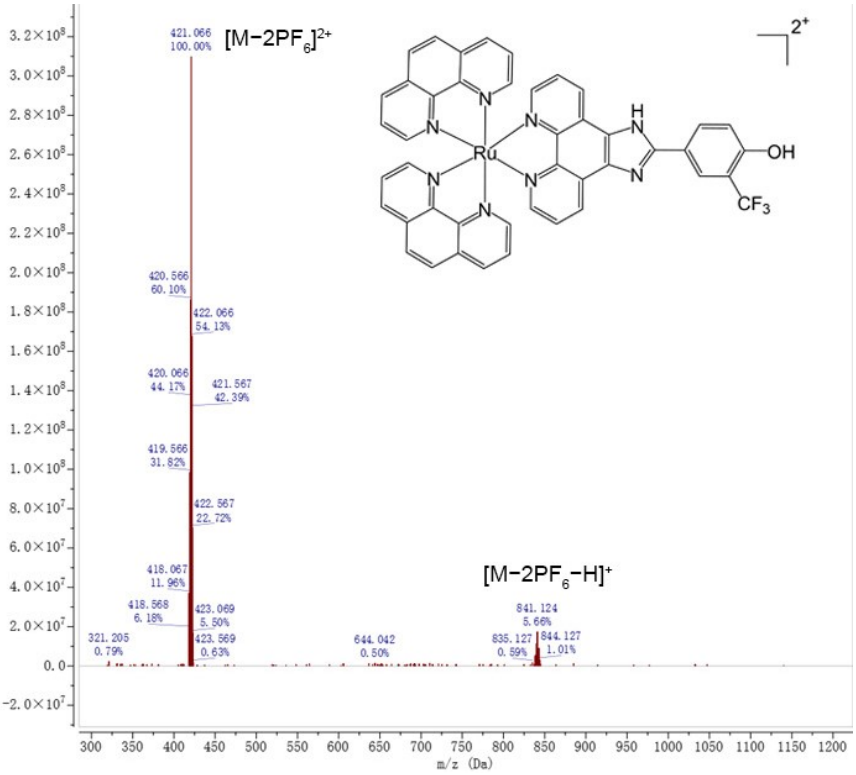


Fig. S10 High-resolution mass spectrometry of the photodegraded product of **Ru2** (5 μM). Irradiation time: 1 h.

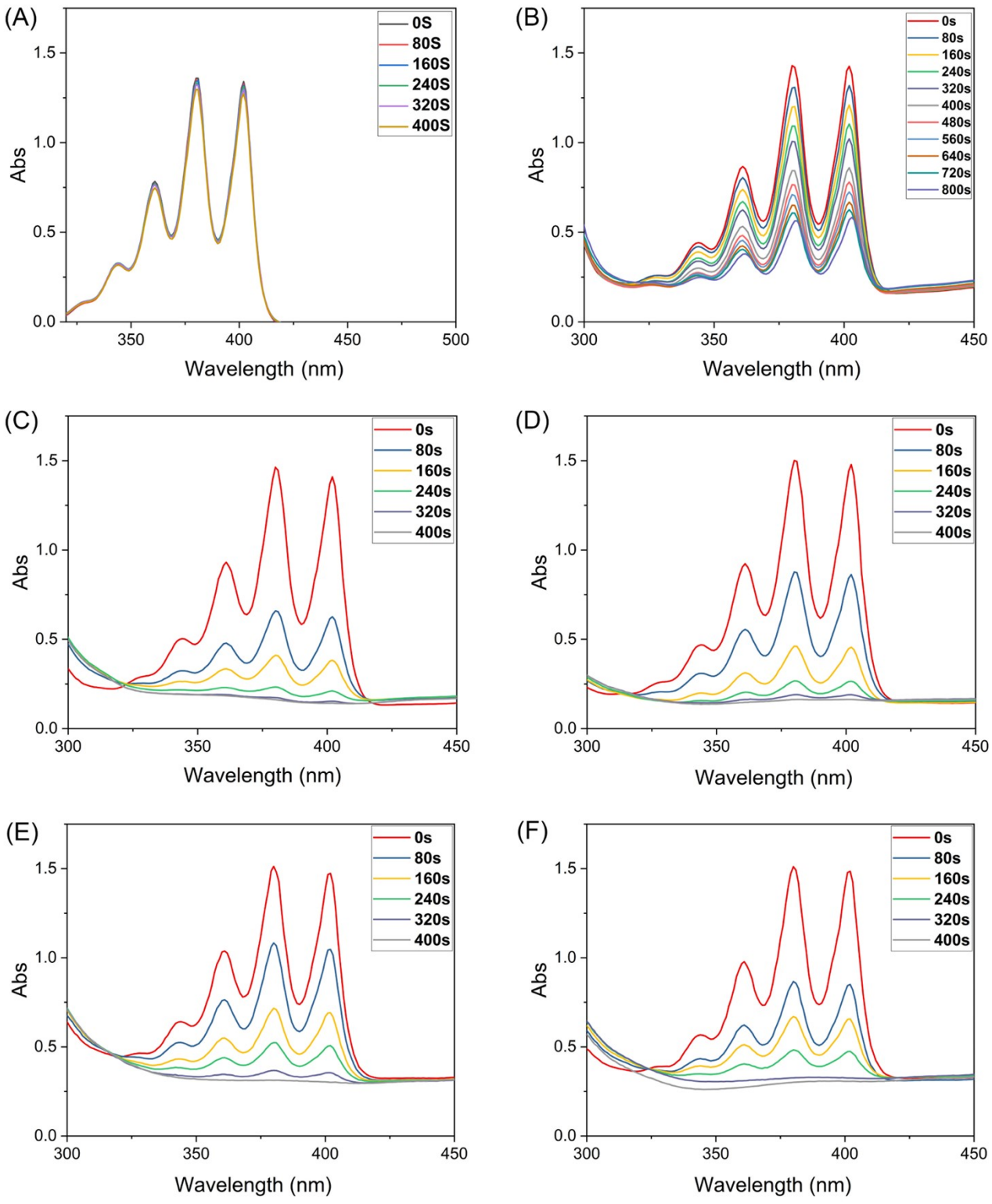


Fig. S11 Photocatalytic oxidation of ABDA upon light irradiation in the presence of (A) control, (B) $[Ru(bpy)_3]Cl_2$ (**Ru-bpy**), (C) **Ru1**, (D) **Ru2**, (E) **Ru1** with PTIO (10 μM), (F) **Ru2** with PTIO (10 μM).

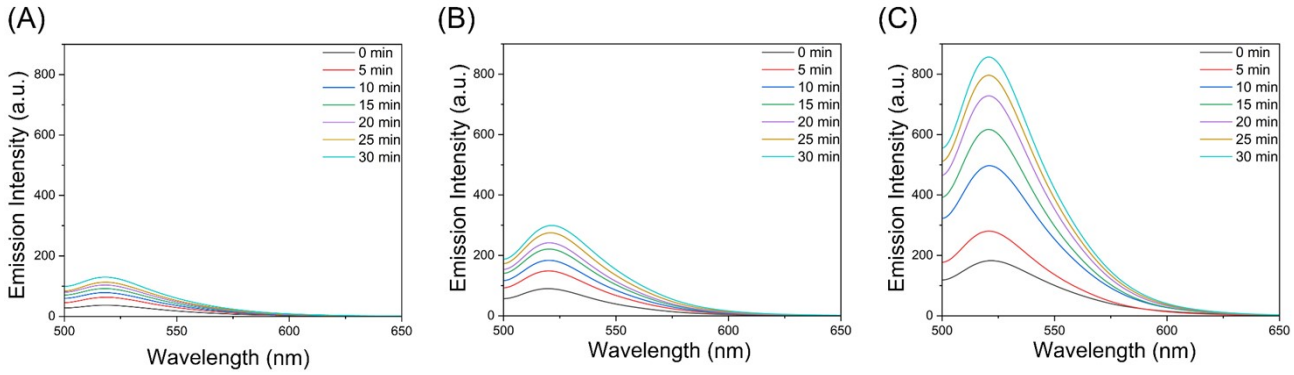


Fig. S12 Fluorescence spectral changes of DAF-FM (10 μM; Ex = 450 nm, Em = 500 - 650 nm) in PBS upon exposure to 425 nm irradiation (5 mW/cm²) in the absence of (A) control, (B) **Ru1** (10 μM), and (C) **Ru2** (10 μM).

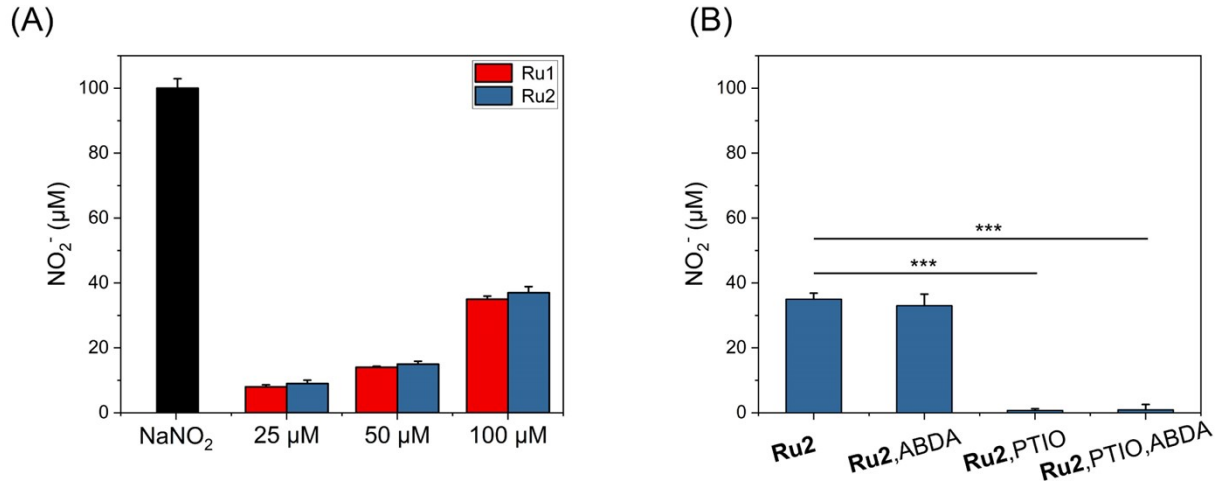


Fig. S13 (A) Photo-induced NO generation from **Ru1** and **Ru2** detected by the Griess assay under irradiation with 425 nm LED light (5 mW/m²) for 30 min. $NaNO_2$ (100 μM) was employed as the standard control. (B) Photo-induced NO generation from **Ru2** (100 μM) in the presence of ABDA (100 μM) or PTIO (100 μM) using the Griess assay. *p<0.05, **p<0.01, ***p<0.001, two-tailed Student's t-test.

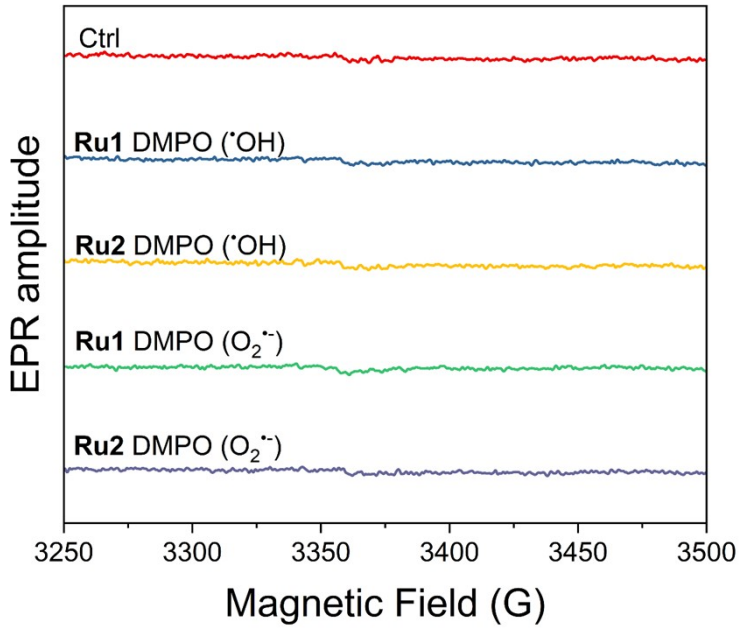


Fig. S14 EPR signals obtained after 425 nm irradiation (5 mW/cm^2, 5 min) of the aqueous solution containing **Ru1** or **Ru2** (40 μM) and DMPO (40 mM).

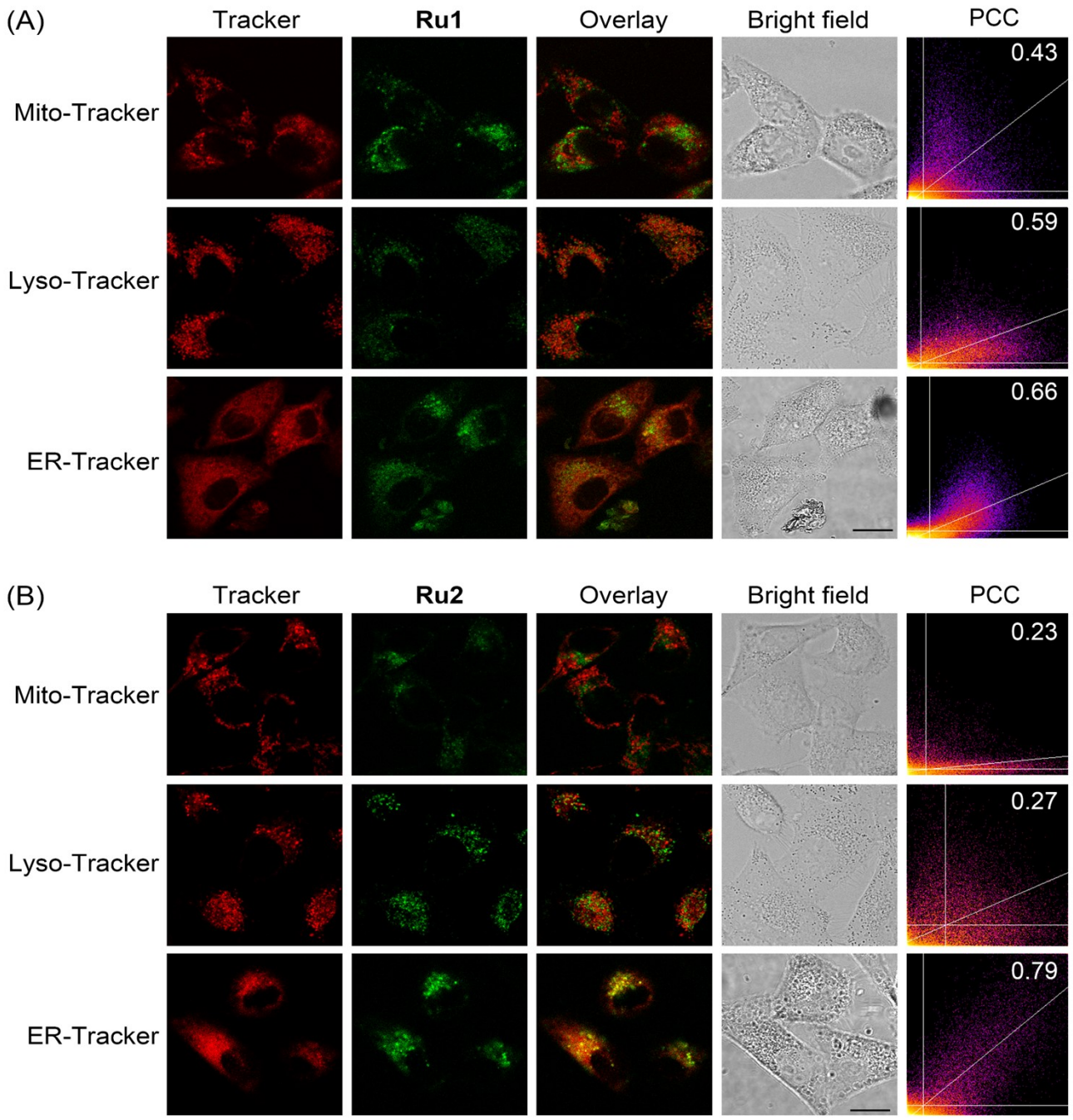


Fig. S15 Confocal co-localization images of HeLa cells treated with (A) **Ru1** or (B) **Ru2** (10 μM, 5 h; Ex = 488 nm, Em = 530 - 570 nm) and then stained with ER-Tracker Red (1.0μM, 30 min; Ex = 552 nm, Em = 600 - 700 nm) or MitoTracker Deep Red FM (150 nM, 30 min; Ex = 633 nm, Em = 670 - 720 nm) or LysoTracker Deep Red (50 nM, 30 min; Ex = 633 nm, Em = 620 - 720 nm). Scale bar, 20 μm.

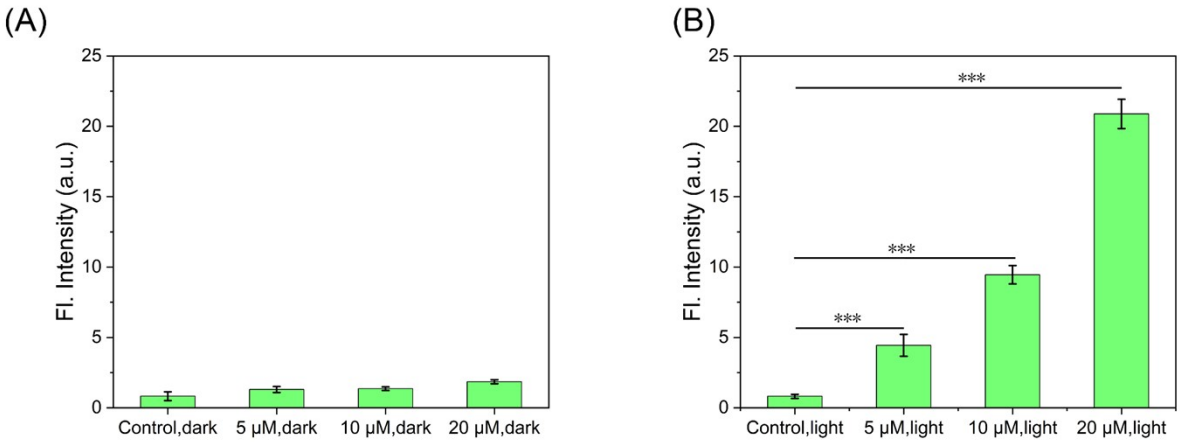


Fig. S16 The fluorescence intensity of DCF in cells. HeLa cells were exposed to varying concentrations of **Ru2** (0, 5.0, 10.0, 20.0 μM) for 12 h and treated with DCFH-DA (10 μM; Ex = 488 nm, Em = 500 - 540 nm) for 20 min. The samples were then kept in darkness (A) or exposed to 425 nm LED light (5 mW/cm^2) irradiation for 30 min (B). *p<0.05, **p<0.01, ***p<0.001, two-tailed Student's t-test.

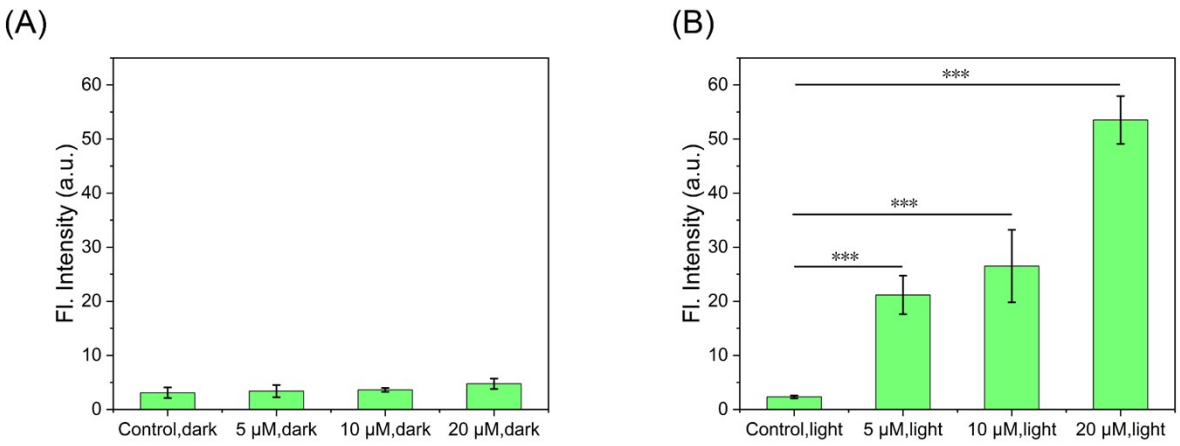


Fig. S17 The fluorescence intensity of DAF-FM in cells. HeLa cells were exposed to varying concentrations of **Ru2** (0, 5.0, 10.0, 20.0 μM) for 12 h and stained with DAF-FM DA (10 μM; Ex = 495 nm, Em = 530 - 570 nm) for 20 min. The samples were then kept in darkness (A) or exposed to 425 nm LED light (5 mW/cm^2) irradiation for 30 min (B). *p<0.05, **p<0.01, ***p<0.001, two-tailed Student's t-test.

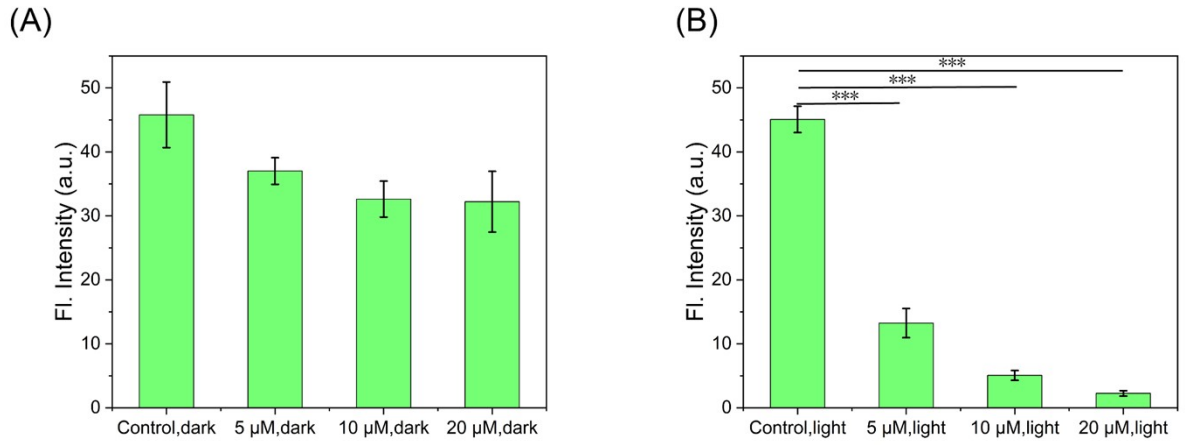


Fig. S18 The fluorescence intensity of Rh 123 in cells. HeLa cells were treated with varying concentrations of **Ru2** (0, 5.0, 10.0, 20.0 μM). Subsequently, the cells were either kept in darkness (A) or exposed to 425 nm LED light (5 mW/cm^2) irradiation for 30 min (B). *p<0.05, **p<0.01, ***p<0.001, two-tailed Student's t-test.

4 References

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