Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

1. General Experimental Details

Unless otherwise noted, all operations were performed without taking precautions to exclude air and moisture. All solvents and reagents were purchased from commercial sources and were used without further purification. Reaction progress was monitored by thin layer chromatography (TLC) using Merck 60 F254 pre-coated silica gel plates illuminating under UV 254 nm or using appropriate stains. Flash column chromatography was carried out using Merck silica gel 70-230 mesh. NMR spectra were measured on Bruker 400 MHz or 500 MHz NMR spectrometer and the chemical shifts were referenced to residual solvent shifts in the respective deutero solvents. Chemical shifts are reported as parts per million referenced with respect the residual solvent peak. HR-MALDI-MS and HR-ESI mass spectra were recorded on a Solarix (Bruker) FTICR-MS. LC-MS analysis was performed on a Shimadzu LC-MS 2020 equipped with an electrospray ionization source and a SPD-20A UV/Vis detector (Shimadzu, Duisburg, Germany).

2. Synthetic Protocol of Ru-SST 8.

$(bpy)_2RuCl_2$ **1** was prepared according to reported protocol.[1] bpy-Alkyne **2** was synthesized in accordance with the literature.[2] Compound 4, 5, 6 and 7 were synthesized according to previous report.[3]

Synthesis of Ru-Alkyne 3

$(bpy)_2RuCl_2$ **1** (0.40 g, 0.83 mmol) was dissolved in 40 ml ethanol/water 3:1 and mixed with 0.15 g (0.83 mmol) bpy-Alkyne **2**. After refluxing for 3 h the residue was purified by sephadex LH-20 column chromatography in methanol. 0.25 g (0.4 mmol) of Ru-Alkyne **3** was isolated as a red powder in 44.6 % yield.[2, 4]

^{1}H NMR (CD_3CN, 400 MHz): δ 8.67 (d, 1H, 3^A, J_{HH} = 1.35 Hz), 8.61 (m, 5H, 3), 8.07 (m, 5H, 4), 7.71 (m, 6H, 6), 7.40 (m, 6H, 5), 4.08 (s, 1H, CCH) ppm. ^{13}C NMR (CD_3OD, 100 MHz): δ 210.1, 159.0, 158.5, 158.4, 158.0, 152.8, 152.7, 152.6, 139.5, 139.4, 133.5, 130.8, 129.3, 129.1, 129.1, 128.0, 126.1, 125.8, 88.6, 80.7 (s, 20C, $C^{aromat.}$), 30.8, 24.3 (s, 2C, ≡C−H, −C≡) ppm. Anal. Calc. for ($C_{32}H_{24}Cl_2N_6Rux4H_2O$): C, 52.18, H, 4.38, N, 11.41. Found: C, 52.57, H, 4.51, N, 11.50.

Synthesis of Ru-SST 8

Azido-SST **7** (10 mg, 4.86 µmol, 1 eq.) and Ru-Alkyne **3** (6.5 mg, 9.78 µmol, 2 eq.) were dissolved in 3 ml of H_2O and followed by adding sodium ascorbate (4 mg, 20.2 µmol, 4 eq.) and copper sulfate (1.6 mg, 10 µmol, 2 eq.) sequentially. The resulting mixture was stirred at RT for 24 h. The mixture was concentrated and purified by Agilent semi-Prep HPLC using an

Agilent Eclipse XDB-C18 Column (9.4 x 250 mm, 5 µm) with the mobile phase starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in acetonitrile), raising to 20% B in 4 min, further raising to 46% B in 12 min and finally reaching 95% B in 5 min, then returning to starting condition 5% B in 5 min and equilibration for 5min with a flow rate of 4 ml/min. The absorbance was monitored at 456 nm and the retention time was 13.21 min and 13.35 min. 8 mg of Ru-SST **8** was obtained from lyophilisation in 61 % yield. HR-ESI-MS: m/z = 530.61507 $[M^{2+}+3H]^{5+}$, 663.01794 $[M^{2+}+2H]^{4+}$ (calcd exact mass: 530.61486 $[M^{2+}+3H]^{5+}$, 663.01670 $[M^{2+}+2H]^{4+}$, formula: $C_{129}H_{158}N_{28}O_{24}RuS_2$). HR-MALDI-MS: m/z = 2649.04410 $[M-H]^+$, 2491.97453 [M-bipyridin-H]$^+$ (calcd exact mass: 2649.04521 $[M-H]^+$, 2491.97486 [M-bipyridin-H]$^+$). LC-MS: m/z = 530 $[M^{2+}+3H]^{5+}$, 663 $[M^{2+}+2H]^{4+}$ (calcd exact mass: 530.61486 $[M^{2+}+3H]^{5+}$, 663.01670 $[M^{2+}+2H]^{4+}$).

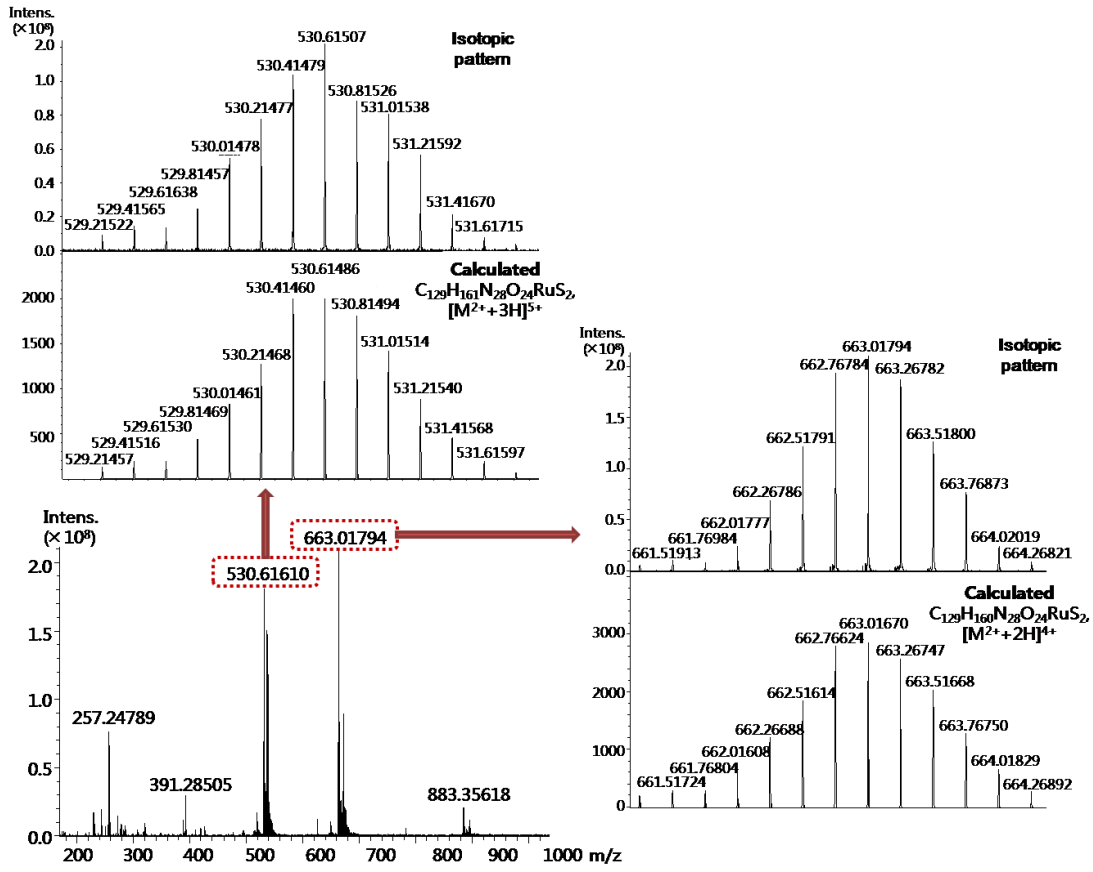

Fig. S1. The HR-ESI-MS spectra of Ru-SST **8**.(calcd exact mass: 530.61486$[M^{2+}+3H]^{5+}$, 663.01670 $[M^{2+}+2H]^{4+}$, formula: $C_{129}H_{158}N_{28}O_{24}RuS_2$).

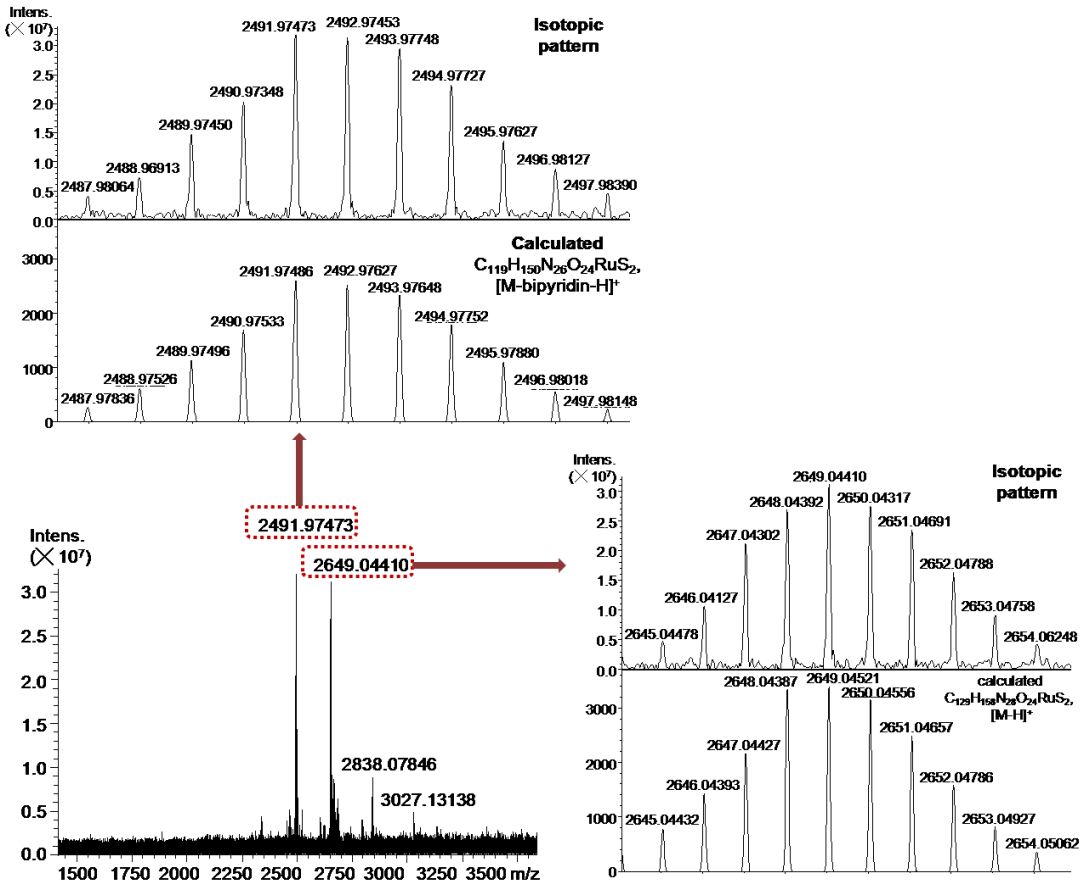

Fig. S2. The HR-MALDI-MS spectra of Ru-SST **8** with α-cyano-4-hydroxycinnamic acid as matrix (calcd exact mass: 2491.97486 [M-bipyridin-H]$^+$, 2649.04521 [M-H]$^+$, formula: $C_{129}H_{158}N_{28}O_{24}RuS_2$).

3. Photophysical Properties.

The UV/Vis absorption spectra were performed with a JASCO Spectrometer V-670. The emission spectra were recorded with a JASCO Spectro-fluorometer FP-8500. All samples were measured in Quartz cuvettes with a path length of 10 mm. All experiments were performed at RT and aerobic conditions if not mentioned otherwise.

Table S1. Absorption bands in MilliQ water (c = 1x10^{-5} mol/L).

	λ_{MLCT} / nm	λ_{LC} / nm	λ_{MC} / nm (shoulder)
Ru-Alkyne**3**	245, 457	287	360, 330
Ru-SST **8**	255, 458	288	360, 330

Table S2. Emission in MilliQ water (c = $1x10^{-5}$ mol/L). The spectra were recorded at λ_{ex} = 460 nm.

	λ_{Emis} / nm	Emis. Intensity / a.u.
Ru-Alkyne**3**	631	94267
Ru-SST **8**	621	158475

4. Photostability.

Photostability tests were performed by monitoring UV/vis absorption in MilliQ water after irradiation with blue LED light (λ = 470 nm, P = 50 ± 3 mW, 50 ± 3 mW/cm^2) for the time intervals 0-300 min. A decay of the characteristic bands was determined (8 % for **Ru-SST 8**, 7 % for Ru-Alkyne **3** and 21 % for $[Ru(bpy)_3]Cl_2$).

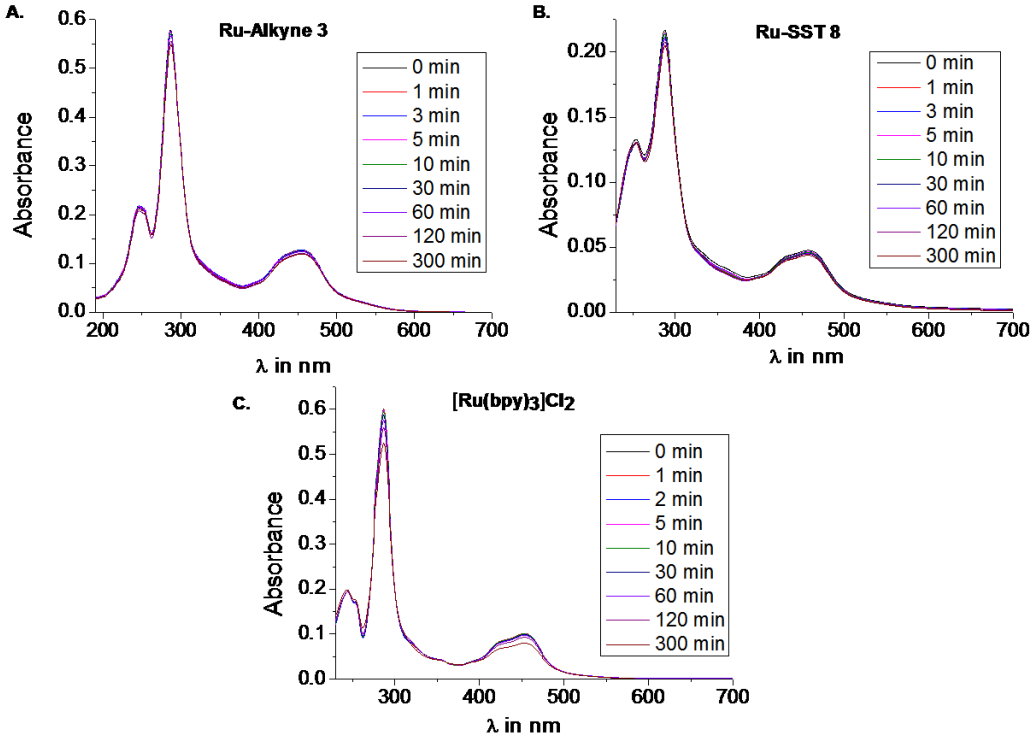

Fig. S3. Photostability of Ru-Alkyne **3** (A), Ru-SST **8** (B) and a typical ruthenium(II) polypyridine $[Ru(bpy)_3]Cl_2$ (C) in MilliQ water (c = $1x10^{-5}$ mol/L).

5. Oxygen Quenching

Emission spectra were recorded both in oxygen free (by flowing argon gas through the solvent for 10 min, p = 0.2 bar) and oxygen saturated MilliQ water. An emission quenching caused by singlet oxygen was obtained around 35 % for Ru-SST **8** and 29 % for Ru-Alkyne **3**.

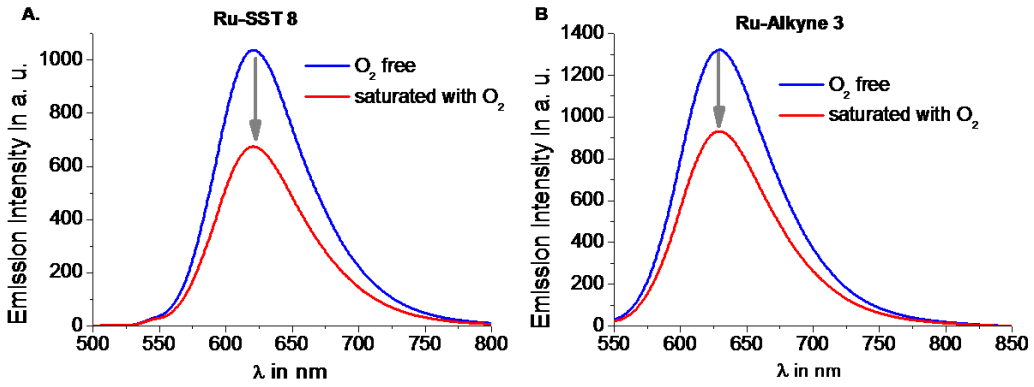

Fig. S4. Emission spectra (λ_{ex} = 460 nm, c = 1x10^{-5} mol/L) of Ru-SST **8** (A) and Ru-Alkyne **3** (B) in MilliQ water saturated with O_2 (red) and O_2 free (blue).

6. Cell Culture.

A549 cells (carcinomic human alveolar basal epithelial cell line) was obtained from DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig) and cultured in DMEM medium with high glucose supplemented and with 10% fetal bovine serum (FBS), 1% 100 U/mL Penicillin, 1% 0.1 mg/mL Streptomycin, 1% 0.1 mM MEM non-essential amino acids at 37°C in a humidified 5% CO_2 incubator.

7. Cellular Uptake Assay.

A549 cells were pre-cultured in high glucose DMEM medium fortified with 10% fetal bovine serum, 1% penicillin/streptomycin and seeded at 6,500 cells/well in a white 96-well (half-area) plate. The cells were left to adhere overnight at 37°C, 5% CO_2. The media was removed and Ru-SST **8** and Ru-Alkyne **3** (dissolved in 50 µl DMEM) were added into each well. The treated cells were subsequently incubated separately for 4 h at 37°C, 5% CO_2. After incubation, the cells were washed (3 times) with Dulbecco's PBS buffer to remove non-specific binding followed by incubating the cells for a further 24 h in 50 µl/well of cell lysis buffer. Emission measurements (λ_{ex} = 456 nm, λ_{em} = 640 nm) were taken using TECAN M1000 microplate reader to determine the uptake efficiencies against non-treated cells as controls. Since the Ru-SST **8** has 1.83 times higher emission than Ru-Alkyne **3**, the emission intensity of Ru-SST **8** in this assay was divided by 1.83.

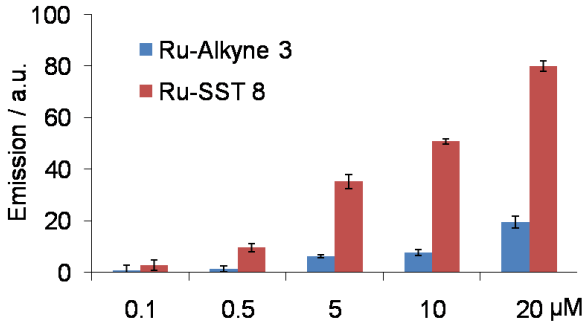

Fig. S5. The cell uptake study of Ru-SST **8** (red) and Ru-Alkyne **3** (blue) at different concentrations (the emission intensity of Ru-SST has been divided by 1.83).

8. Cellular Uptake Monitored by Laser Scanning Confocal Microscopy.

A549 cells were seeded at a density 30,000 cells/well in a μ-Slide 8-well chambered cover slip (ibidi, Martinsried, Germany) in 300 μl DMEM medium. The cells were cultured overnight to allow adhesion at 37°C, 5% CO_2. Subsequently, the medium was removed and 10 μM of Ru-Alkyne **3** and 10 μM of Ru-SST **8** were added respectively. The cells were then further incubated for 4 h in the incubator at 37°C, 5% CO_2. Before imaging, cells were washed with DMEM medium for 3 times. The live cell imaging was performed using a LSM 710 laser scanning confocal microscope system (Zeiss, Germany) coupled to an XL-LSM 710 S incubator and equipped with a 63x oil immersion objective. The emission of the Ru complex was recorded using a 580-707 nm filter and a 458 nm Argon laser for excitation. The acquired images were processed with ZEN 2011 software.

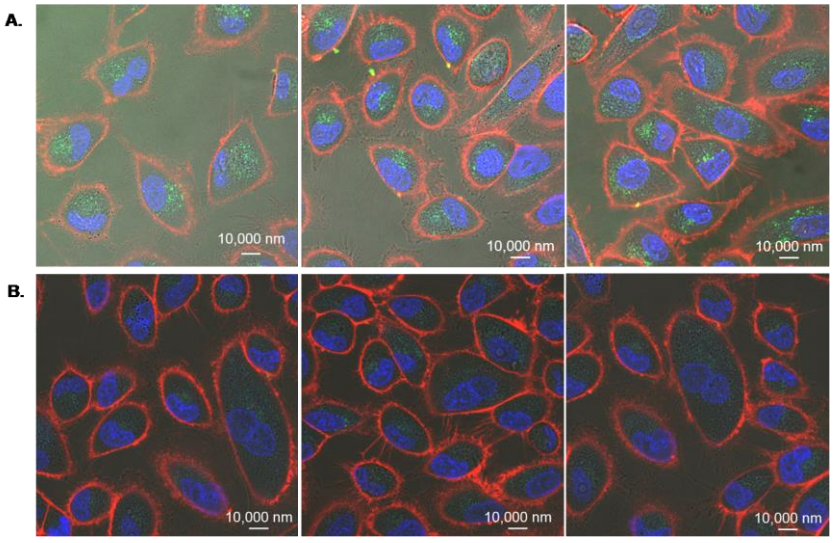

Fig. S6. Confocal microscopy images of 10 μM Ru-SST **8** (A) and Ru-Alkyne **3** (B) incubated with A549 cells for 4h.

9. Phototoxicity.

A549 cells were pre-cultured in high glucose DMEM medium with 10% fetal bovine serum, 1%

penicillin/streptomycin without phenol-red and seeded at 6,500 cells/well in a white 96-well (half-area) plate. The cells were left to adhere overnight at 37°C, 5% CO_2. The media was removed and different concentrations of Ru-SST **8 or** Ru-Alkyne **3** (dissolved in 50 µl DMEM) were added into each well. The treated cells were subsequently incubated for 4 h at 37°C, 5% CO_2. After incubation, the cells were washed and irradiated by a 470 nm LED array (P = 23 ± 3 mW for 5 min, 6.9 ± 0.9 J/cm^2). The cells were further incubated for 6 h in the dark. As for the dark control assays, the plate was kept in the dark during the whole process. After washing, the cells were treated with Tox-8 reagent. After 2 h incubation, the emission intensity was measured by a Tecan Infinite M1000 microplate reader (λ_{ex} = 570 nm, λ_{em} = 590 nm). The wells without cells but with Tox-8 reagent were used as controls. Each experiment was performed in triplicates. The cell viability rate (VR) was calculated according to the following equation:

$$VR = [(A-C)/(B-C)]\times 100\%$$

Where A is the average emission of experimental groups with the treatment of the compound, B is the average emission of the experimental groups without the treatment of the compound, and C is the average emission of the culture medium background. IC_{50} values for cytotoxicity were calculated by Graph Pad Prism 5.

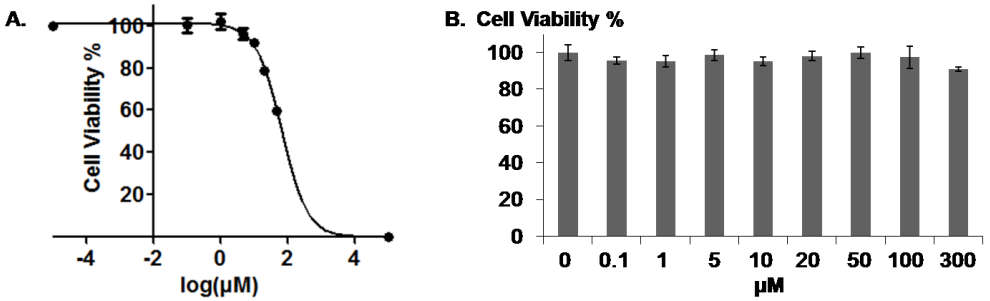

Fig. S7. A. Cytotoxicity of Ru-Alkyne **3** on A549 cells with light irradiation for 5 min (IC_{50} = 67.5 ± 1.1 µM). B. Cytotoxicity of Ru-Alkyne **3** on A549 cells in the absence of light.

The toxicity of light source has also been determined by the cell viability assay described above. It has shown that 98.5 ± 5.1 % of cell viability upon light irradiation, indicating that the light source is not harmful to the cells (Fig. S8).

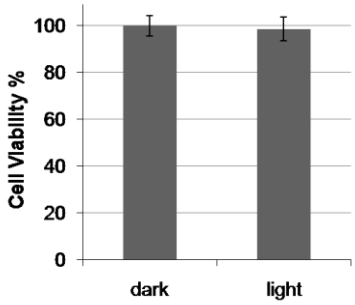

Fig. S8. Cell viability assay on the A549 cells with or without light for the determination of the toxicity caused by light.

10. Reference

[1] S. Rau, B. Schäfer, A. Grüßing, S. Schebesta, K. Lamm, J. Vieth, H. Görls, D. Walther, M. Rudolph, U. W. Grummt, E. Birkner, *Inorg. Chim. Acta* **2004**, *357*, 4496.

[2] A. Baron, C. Herrero, A. Quaranta, M.-F. Charlot, W. Leibl, B. Vauzeilles, A. Aukauloo, *Inorg. Chem.* **2012**, *51*, 5985.

[3] T. Wang, Y. Wu, S. L. Kuan, O. Dumele, M. Lamla, D. Y. Ng, M. Arzt, J. Thomas, J. O. Mueller, C. Barner-Kowollik, T. Weil, *Chem. Eur. J.* **2015**, *21*, 228.

[4] a)R. R. Allison, C. H. Sibata, *Photodiagn. Photodyn.* **2010**, *7*, 61; b)H. T. Ratte, *Environ. Toxicol. Chem.* **1999**, *18*, 89.