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In-vitro Fluorescence and Phototoxicity of *ß*-$SnWO_4$ Nanoparticles

– SUPPORTING INFORMATION –

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1. Analytical tools

Scanning electron microscopy (SEM) was carried out with a Zeiss Supra 40 VP microscope. Diluted aqueous suspensions of as-prepared *β*-$SnWO_4$ were deposited on silicon wafers and evaporated. The acceleration voltage was in the range of 5-10 kV and the working distance was 3 mm.

Dynamic light scattering (DLS) was conducted with polystyrene cuvettes applying a Nanosizer ZS from Malvern Instruments.

Zeta-potential measurements were performed in polystyrene cuvettes applying a Nanosizer ZS from Malvern Instruments. To this purpose, aqueous suspensions were titrated from neutral to alkaline and acidic pH by addition of NaOH and HCl, respectively.

Diffuse reflectance spectra (UV-VIS) of powders were recorded in a wavelength interval of 250–800 nm with a Varian Cary 100 spectrometer, equipped with an integrating sphere against $BaSO_4$ as a reference.

Cell culture and artificial daylight illumination: All cells were cultured at 37 °C, 5 % CO_2 and moisture atmosphere. To exclude environmental effects on both cell growth and viability, artificial daylight illumination was performed under equal conditions. For short time illumination, cell samples were transferred to a glass front incubator and exposed to daylight from the front side. For 24 h illumination, white-light LED lamps were installed inside the incubator (3 lamps of 4 LEDs, 0.5 W each, colour temperature 15000–19000 K (cool white), luminous intensity 14–18 cd). Samples for illumination were deposited 10 cm below.

2. Synthesis of the *β*-$SnWO_4$ nanoparticles

Nanoscaled *β*-$SnWO_4$ was synthesized by precipitation utilizing electrostatic stabilization. In a typical recipe, $SnCl_2$ (417.1 mg, 2.2 mmol) was dissolved in 2.5 mL of carbonated water. Under vigorous magnetic stirring, this solution was added to 2.5 mL of an equimolar aqueous solution of $Na_2WO_4 \cdot 2H_2O$ (725.7 mg) at ambient temperature. A bright yellow nanomaterial was generated immediately and the resulting suspension was directly diluted with 20 mL of phosphate-buffered saline (PBS: 8.0 g/L NaCl; 0.2 g/L KCl; 1.42 g/L Na_2HPO_4; 1.78 g/L $Na_2HPO_4 \cdot 2H_2O$; 0.27 g/L KH_2PO_4). The obtained nanomaterial was collected by centrifugation (15 min, 25,000 rpm), redispersed two times in PBS and centrifuged again for washing. Finally, the washed suspension was centrifuged for 2 min at 10,000 rpm in

order to remove agglomerates. In order to improve membrane penetration and cell uptake, the as-prepared β-$SnWO_4$ nanoparticles, moreover, can be coated with protamine (i.e. protamine sulfate from herring).

The obtained centrifugate represents an aqueous suspension of β-$SnWO_4$ nanoparticles showing diameters well below 20 nm and a mean diameter of 8(±2) nm (0.13 wt-%, 45% yield of dry powder) being fairly stable without any stabilizing agents (*cf. main text: Figure 1*). Note that only biocompatible solvents were used with PBS having the same pH (pH = 7.4) and the same osmotic pressure than human blood.

3. UV-Vis spectra and optical absorption of the β-$SnWO_4$ nanoparticles

UV-Vis spectra show the optical properties of the as-prepared β-$SnWO_4$ nanoparticles (Figure S1). The spectra show a steep absorption with its onset at 550 nm for β-$SnWO_4$. The optical band gap was determined from a Tauc-plot and resulted in 2.7 eV for β-$SnWO_4$.[1] Figure S1A displays the absorbance spectra of the as-prepared β-$SnWO_4$ nanoparticles, which was derived from the diffuse reflectance spectra according to the Kubelka-Munk equation. The optical band gap was determined from a Tauc-plot (Figure S1B): The linear region in the $(\alpha hv)^2$-*versus*-hv plot was fitted. The obtained value of 2.73 eV for the band gap of β-$SnWO_4$ is in good agreement with previously reported values of the bulk-compound (2.68 eV, 2.6 eV).[2]

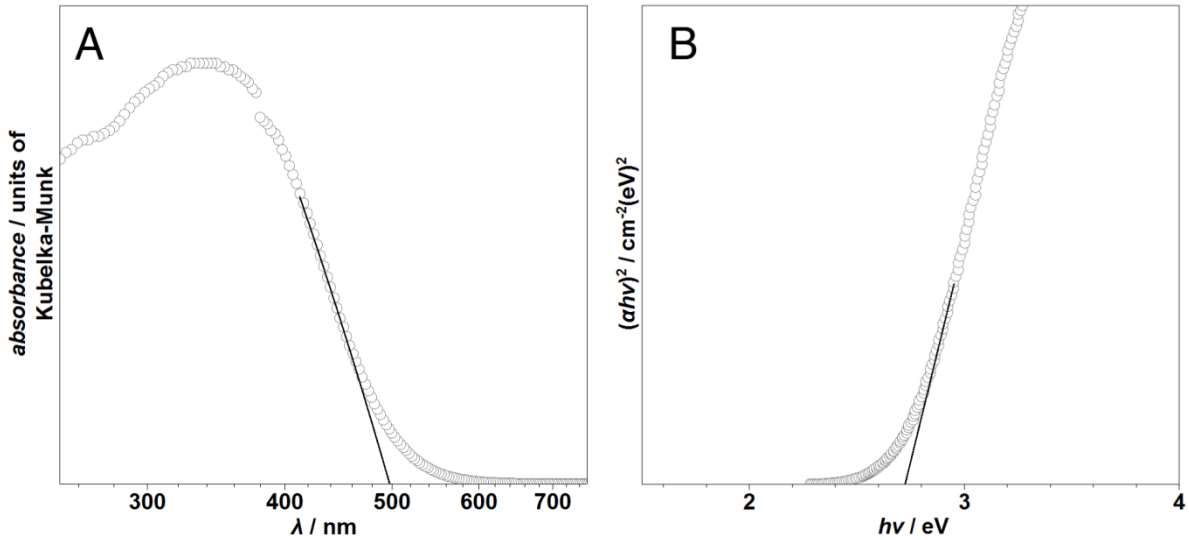

Figure S1. (**A**) UV-Vis diffuse reflectance spectra of the as-prepared β-$SnWO_4$ nanoparticles; (**B**) Tauc plot for optical band-gap determination.

4. *In-vitro* cytotoxicity of *β*-$SnWO_4$ nanoparticles in HepG2 and HeLa cells

The influence of the *β*-$SnWO_4$ nanoparticles on cell proliferation was quantified via cell count experiments. 2×10^4 HepG2 or HeLa cells/ml were seeded into a 24 well plate and cultivated at 37 °C and 5 % CO_2. The following day, samples were exposed to *β*-$SnWO_4$ and/or illumination or not treated at all (triplicates, $n = 3$). 24, 48 and 72 hours later, cells were harvested with trypsin/EDTA and counted.

As shown in Figure S2, stagnation in cell proliferation is observed when cells were exposed to *β*-$SnWO_4$ nanoparticles and illumination, whereas HepG2 cells proliferate normally when incubated in the dark. These results are in accordance to MTT studies (*cf.* Figures S6, S7; *cf.* main text: Figures 4,5). Treatment with *β*-$SnWO_4$ in combination with light clearly hampers the proliferation of HepG2 cells whereas proliferation rates do not stagnate if the cells are kept in the dark. To investigate, whether these results could be confirmed on a subcellular level, we tested the influence of *β*-$SnWO_4$ nanoparticles on mitochondrial integrity both with and without light exposure.

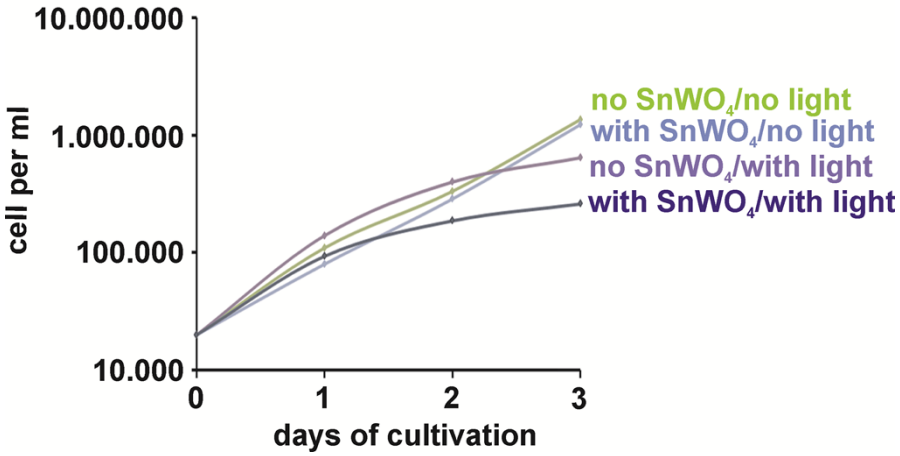

Figure S2. Proliferation of HepG2 cells in the presence and absence of *β*-$SnWO_4$ nanoparticles (10 µM), either cultivated in the darkness or in light.

A significant decrease in mitochondrial integrity was observed when HepG2 cells were exposed to both *β*-$SnWO_4$ nanoparticles and light (Figure S3). Due to the treatment of HepG2 cells with *β*-$SnWO_4$, ROS were generated in mitochondria. Increasing concentration of ROS in the mitochondria results in an increased fusion and fission of mitochondria and their microtubule associated transport towards the nucleus. The round-shaped mitochondria accumulated in the perinuclear region and the release of ROS triggers apoptosis and necrosis. Finally, ROS initiate a cascade of radical reaction, damage the cell irreparably, and determine cell membrane destabilisation and ultimately cell death.[3] Similar results could be observed in HeLa cells (Figure S4).

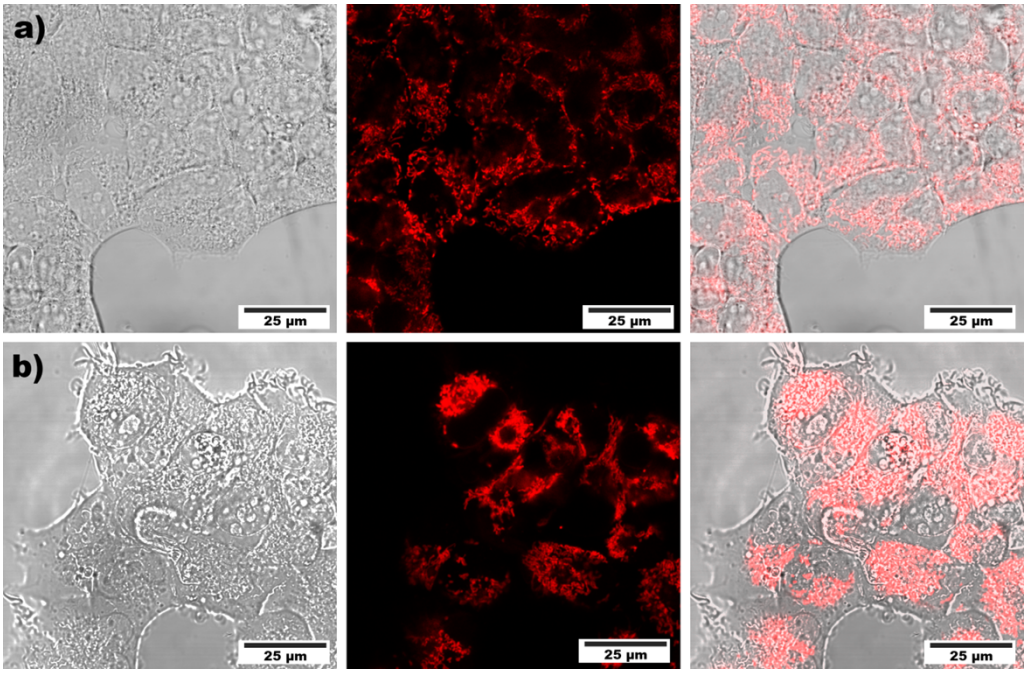

Figure S3. Mitochondrial integrity after treatment of HepG2 cells with β-$SnWO_4$ nanoparticles (10 µM). Cells were either kept in the dark (a) or illuminated for 24 hours (b) before staining with MitoTracker® Deep Red FM (life technologies™). Light induced reorganization of mitochondria, loss of mitochondrial integrity and formation of filopodia indicates early stages of apoptosis whereas cells cultivated in the dark show equal distribution of mitochondria throughout the cell.

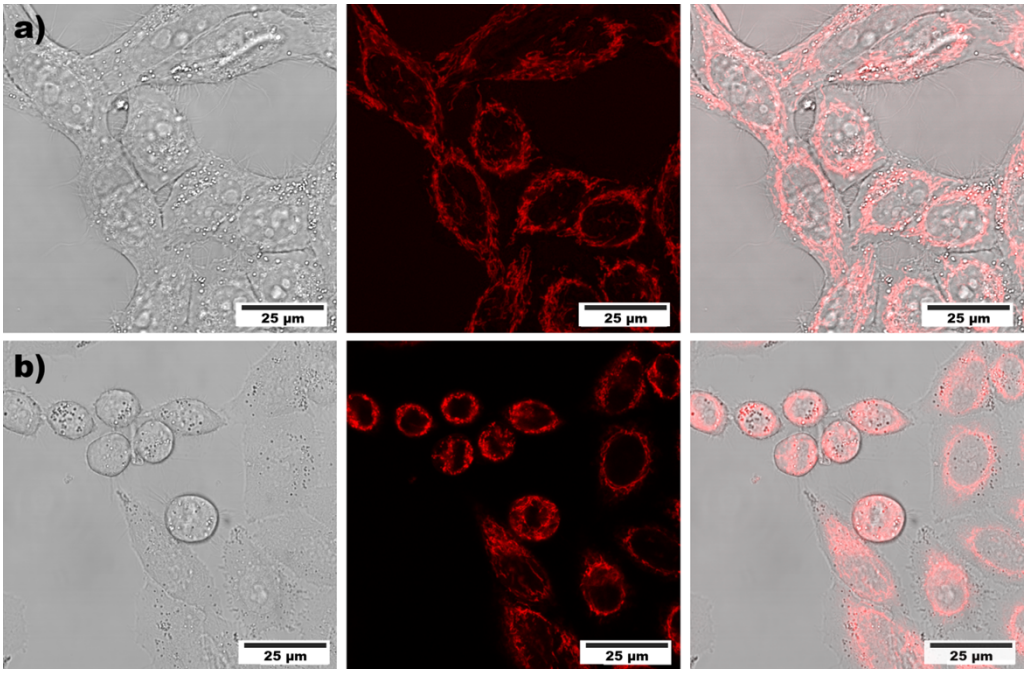

Figure S4. Mitochondrial integrity after treatment of HeLa cells with β-$SnWO_4$ nanoparticles (10 µM). Cells were either kept in the dark (a) or illuminated for 24 hours (b) before staining with MitoTracker® Deep Red FM (life technologies™). Light induced reorganization of mitochondria and loss of mitochondrial integrity indicates early stages of apoptosis whereas cells cultivated in the dark show equal distribution of mitochondria throughout the cell.

MTT assays were performed to determine the cell viability. The yellow tetrazolium compound 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide used in this assay was reduced to purple formazan by mitochondrial enzymes and quantified photometrically. As this reaction was limited to metabolically active cells, the amount of formazan directly correlated with the cell viability. HepG2 cells were seeded on 96-well plates at a density of 1×10^4 cells/well and cultivated at 37 °C and 5 % CO_2. Cells were incubated with β-$SnWO_4$ nanoparticles in diverse concentrations for 1–3 days. While reference samples were not illuminated, other samples of cells were illuminated with daylight either frequently or only once three days after transfection. Controls were treated with 5 µl Triton X-100. Afterwards, 15 µl of MTT solution (dye solution for MTT test, Promega) were added to each well and incubated for 1.5 hours. The reaction was stopped by addition of 100 µl of lysis buffer. 1.5 hours later, the absorbance of the converted dye was measured in a photometer (ultra microplate reader ELx808, BioTEK Instruments) at a wavelength of 595 nm.

The *in-vitro* cytotoxicity of the as-prepared β-$SnWO_4$ nanoparticles was evaluated after incubation in HepG2 and in HeLa cells in the dark and after illumination using an MTT assay. The low systemic cytotoxicity when keeping in the dark only, the acute killing potency against HepG2 and HeLa cells if illuminated 2 hours after transfection, and the negligible long-term effect 72 h after transfection are shown in the main text (Figure S5, *cf. main text: Figure 4*). The influence of increasing the incubation time of the HepG2 cells with the β-$SnWO_4$ nanoparticles from 24 h to 48 h is shown in Figure S6. Again an acute phototoxic effect proportional to the dose of β-$SnWO_4$ is visible only for daylight illumination right after transfection (Figure S6: red bars). If only kept in the dark (Figure S6: dark green bars) or if illuminated after 72 h in the dark (Figure S6: light green bars), the β-$SnWO_4$ nanoparticles did not cause any appreciable cytotoxic effect in MTT assays nor any negative long-term effect. Cells treated with 0.5 to 10 µM of β-$SnWO_4$ in the dark or cells illuminated after 72 hours after transfection, independent of dose and duration of treatment show a survival rate of around 100 % and a LD50 > 10 µM within the statistical error, demonstrating an excellent biocompatibility.

When comparing the phototoxic effect of the β-$SnWO_4$ nanoparticles on HepG2 cells (*cf. main text: Figure 4*) and on HeLa cells (Figure 5), the effect is clearly visible for both types of cells but more pronounced for HepG2 cells. This can be ascribed, on the one hand, to the different types of cells having, for instance, different membrane permeability and metabolic activity. On the other hand, the here applied concentrations of β-$SnWO_4$ nanoparticles and periods of illumination were optimized for HepG2 cells. These conditions were exactly similarly used for HeLa-cell treatment in order to allow for direct comparison. For optimal treatment of HeLa cells, the experimental conditions would have needed a specific optimization different than for HepG2 cells.

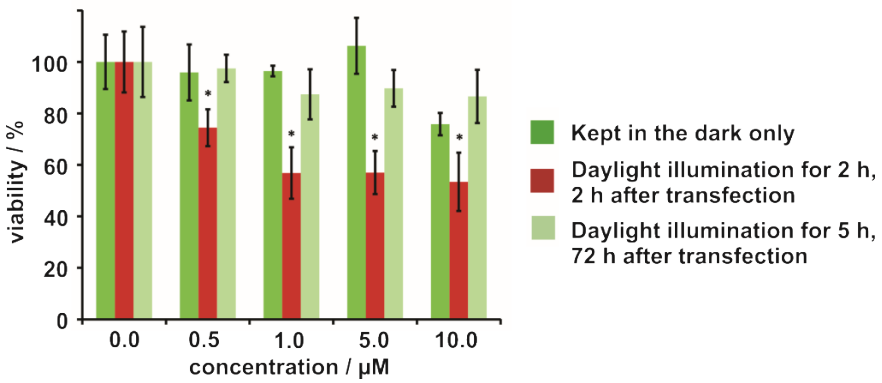

Figure S5. Evaluation of the *in-vitro* cytotoxicity of *β*-$SnWO_4$ nanoparticles in HeLa cells in the dark and after illumination using MTT assays. Cells were incubated with the nanoparticles for 72 hours as described in Figure 4 (*cf. main text*). Statistical error bars were calculated from triplicates of $n = 3$.

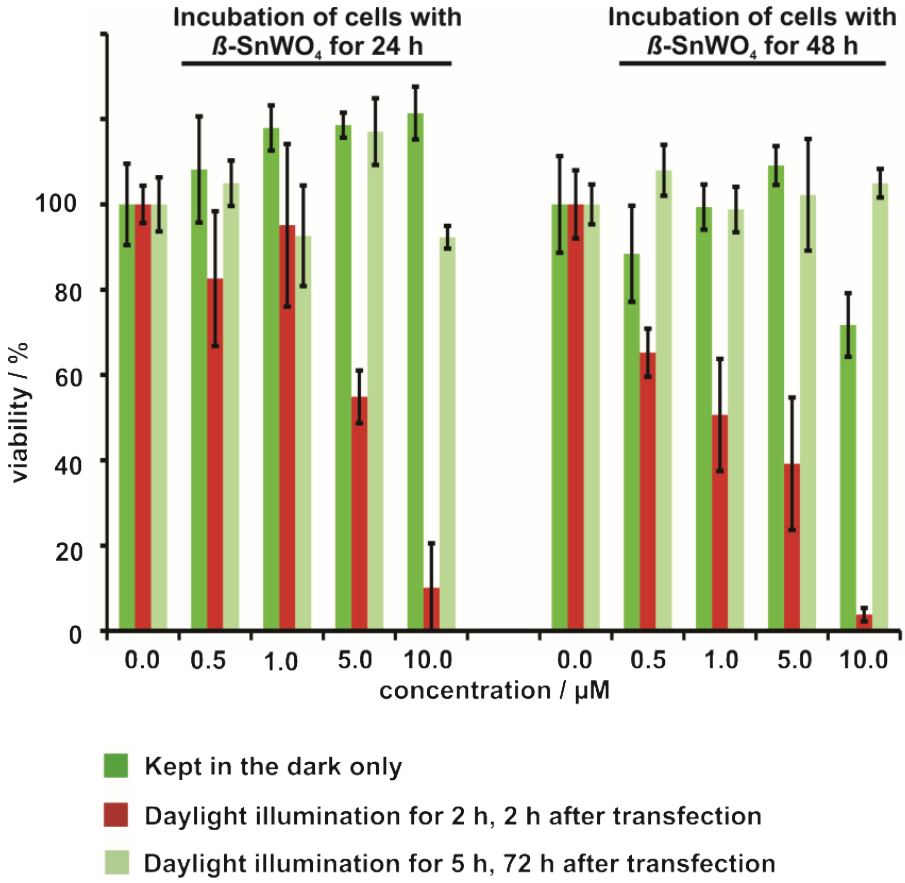

Figure S6. Evaluation of the *in-vitro* cytotoxicity of *β*-$SnWO_4$ nanoparticles in HepG2 in the dark and after illumination using MTT assays. Cells were incubated with the nanoparticles for 24 or 48 hours, respectively, as described in Figure 4 (*cf. main text*). Statistical error bars were calculated from triplicates of $n = 3$.

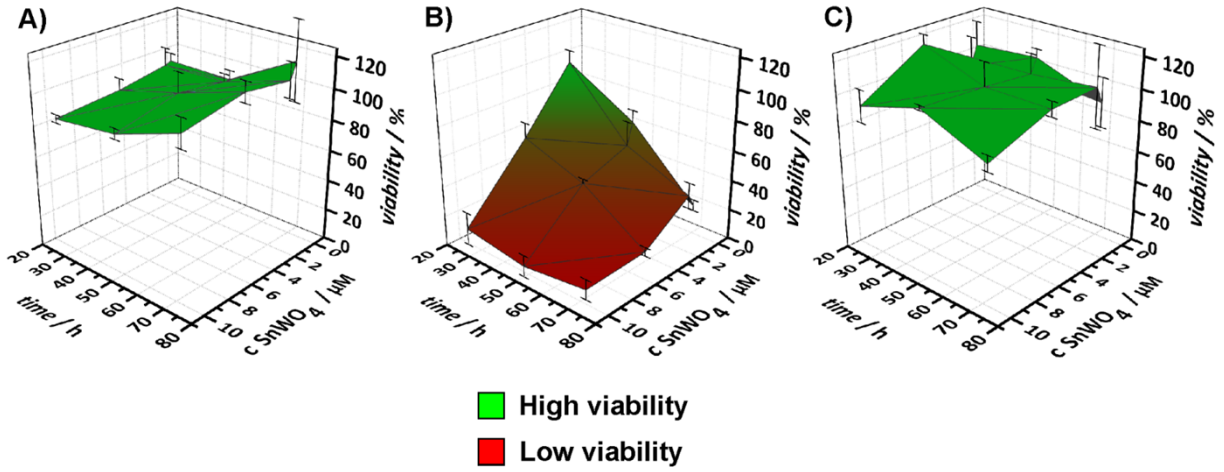

Figure S7. Evaluation of the *in-vitro* cytotoxicity of the β-$SnWO_4$ nanoparticles in HepG2 cells using MTT assays: (**A**) Kept in the dark only; (**B**) Reiterated daylight illumination (2 h of illumination every 24 h, beginning 2 h after transfection); (**C**) Illumination for 5 h after 72 h of transfection. Cells were incubated with the nanoparticles for 24 hours as described in Figure 4 (*cf. main text*).

To estimate the dose-response relationship, the β-$SnWO_4$ nanoparticles were applied in four different concentrations (0.5 µM, 1.0 µM, 5.0 µM, 10 µM). Moreover, the cell viability was examined with MTT assays after 24 h, 48 h, and 72 h, respectively. Altogether, this results in 4 times 3 pairs of values (Figure S7). These set of values has been collected three times for HepG2 cells that were:

(i) Only kept in the dark after transfection (Figure S7A)

(ii) Illuminated with daylight for 2 h daily, beginning 2 h after transfection (Figure S7B, *cf. main text: Figure 5*)

(iii) Kept in the dark for 72 h after transfection and illuminated thereafter with daylight for 5 h (Figure S7C)

The results for these three sets of MTT assays are displayed in Figure S7. Accordingly, β-$SnWO_4$ nanoparticles did not show any significant cytotoxicity in the dark (Figure S7A), demonstrating an excellent biocompatibility and a low systemic toxicity: In the dark, transfected HepG2 cells show 100% viability within the statistical error limits independent of the dose of β-$SnWO_4$ nanoparticles and the duration of the experiment (Figure S7A). If the HepG2 cells were illuminated 2 hours after transfection (Figure S7B), however, the β-$SnWO_4$ nanoparticles display an acute cell-killing potency proportional to its dose and the incubation time (Figure S7B, *cf. main text: Figure 5*). Finally, the β-$SnWO_4$ nanoparticles were also not cytotoxic if illuminated only at the third day (for 5 h) after transfection (Figure S7C), again exhibiting 100% viability within the statistical error limits, independent of dose and duration. Thus, there is no undesired long-term effect accompanied with the nanoparticles.

References

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