SUPPORTING INFORMATION

Differential Behaviour of Cationic Triphenylamine Derivatives in Fixed and Living Cells

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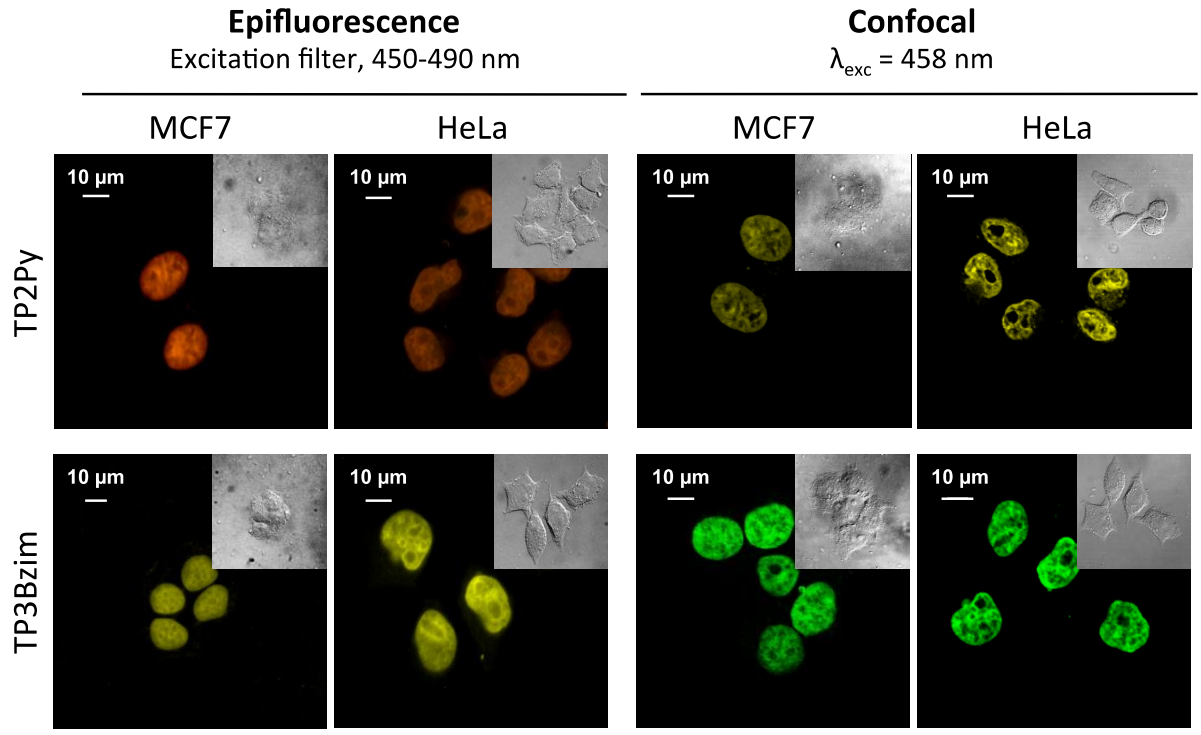

Fig. S1 Subcellular localization of TPAs in fixed cells. Epifluorescence (left) and confocal (right) imaging of fixed MCF7 or HeLa cells treated with **TP2Py** (top) or **TP3Bzim** (bottom). Cells were first fixed with 4% paraformaldehyde and then incubated with 2µM TPA for 2h (fixation of cells with ethanol led to similar results). The emission filter for epifluorescence was 515-700 nm. The emission slit setting for confocal imaging was 560-720 and 530-690 nm for **TP2Py** and **TP3Bzim**, respectively. Insets: corresponding DIC (differential interference contrast) transmission images.

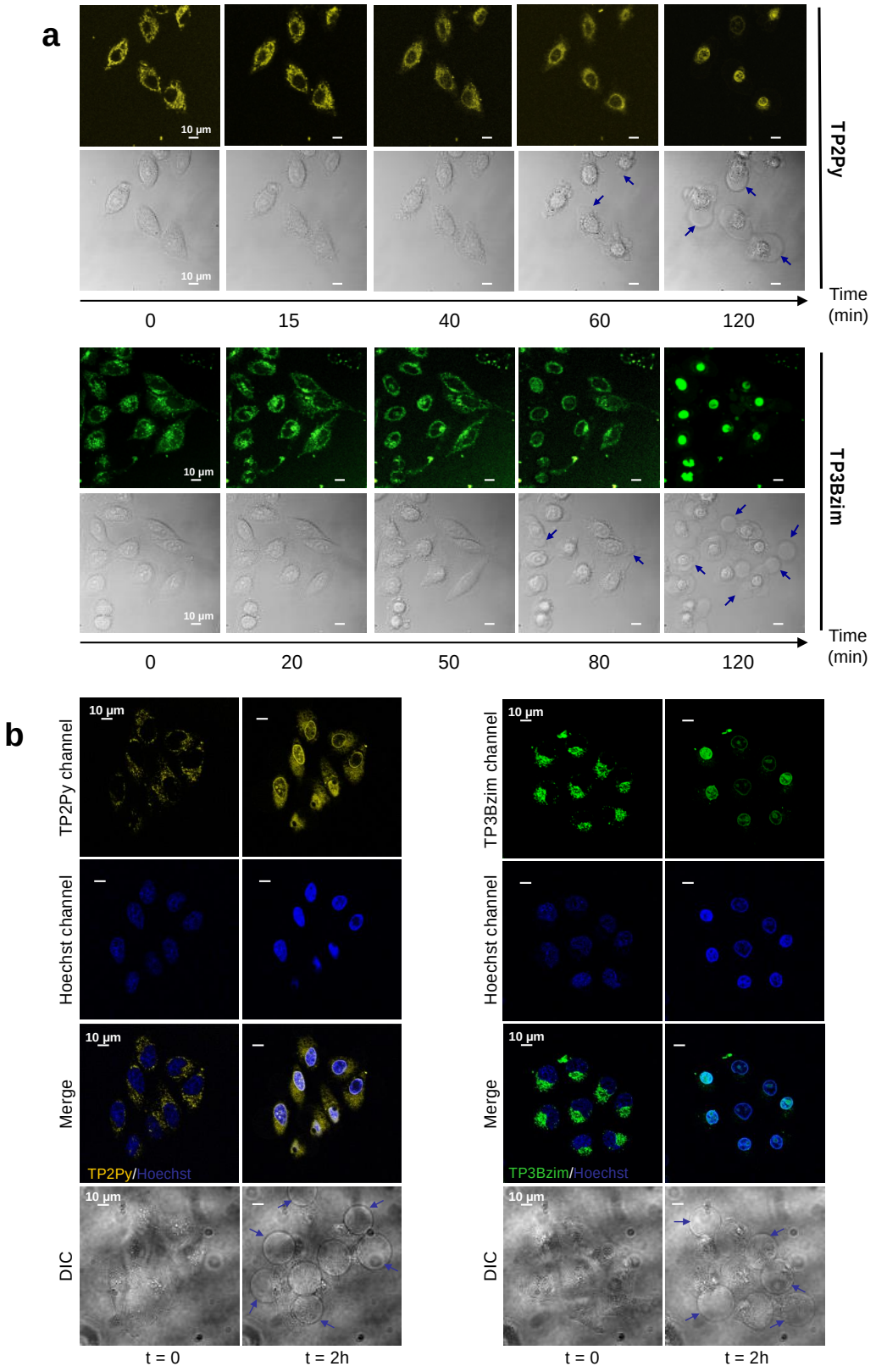

Fig. S2 Confocal fluorescence imaging of living HeLa cells treated with TPAs. (a) The fluorescence signal of TPAs (top: **TP2Py**; bottom: **TP3Bzim**) is mainly localized in the cytoplasm at t = 0 and relocalizes to the nucleus under light illumination (λ_{exc} = 458 nm; irradiance, 30 mW/cm^2) in a similar manner to that observed for MCF7 cells (Fig. 1). HeLa cells were pre-incubated with 2µM TPA for 2h at 37°C before irradiation. (b) Similar experimental conditions as shown in panel a except that HeLa cells were pre-incubated with 2µM TPA (left: **TP2Py**; right: **TP3Bzim**) and 1µM Hoechst 33342. Excitation wavelengths of TPAs and Hoechst were 458 and 750 nm, respectively. Emission slit settings: 560-720, 530-690 and 406-506 nm for **TP2Py**, **TP3Bzim** and Hoechst, respectively. Merge: After 2h irradiation (2nd column), white and cyan areas indicate colocalizations of **TP2Py**/Hoechst and **TP3Bzim**/Hoechst, respectively. Corresponding DIC transmission images illustrating the concomitant membrane blebbing (blue arrows) are shown below the fluorescence images.

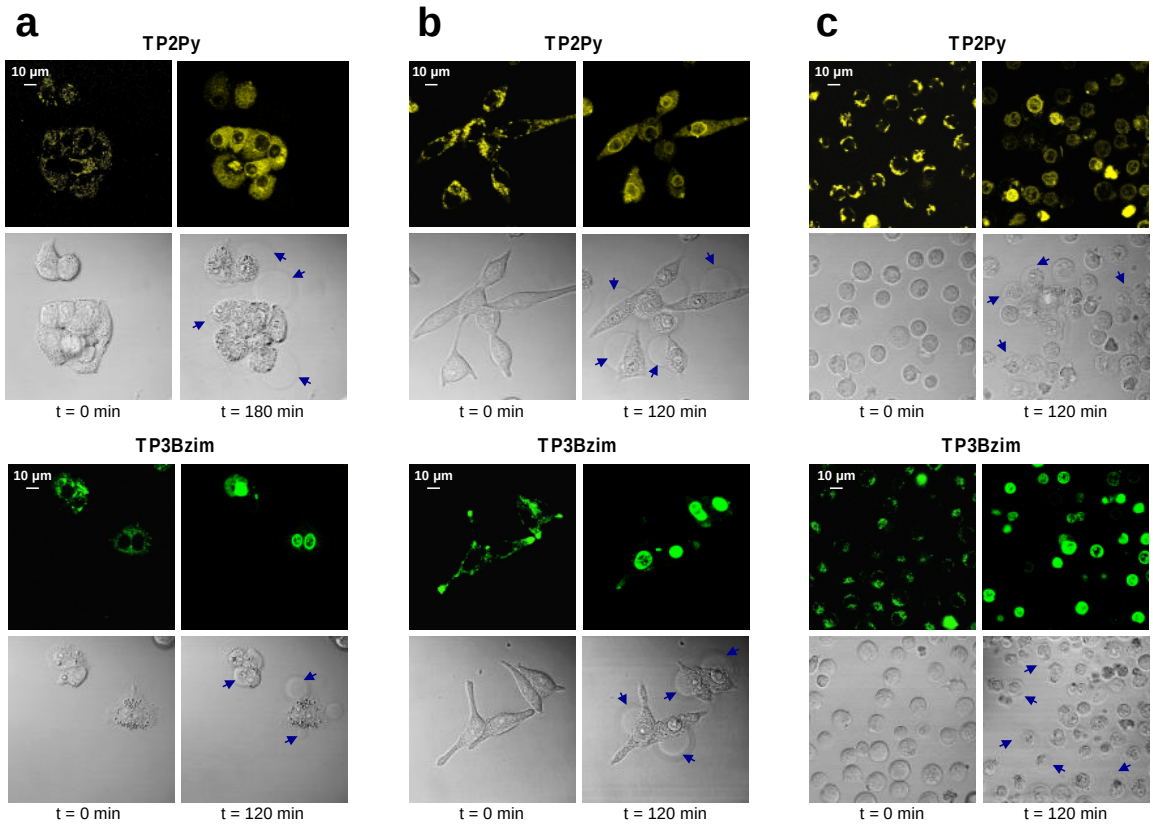

Fig. S3 Confocal fluorescence imaging of living HepG2 (a), LNCaP (b) and Jurkat (c) cells treated with TPAs. The fluorescence signal of TPAs (top: **TP2Py**; bottom: **TP3Bzim**) is mainly localized in the cytoplasm at t = 0 and relocalizes to the nucleus under light illumination (λ_{exc} = 458 nm; irradiance, 30 mW/cm^2) in a similar manner to that observed for MCF7 (Fig. 1) and HeLa cells (Fig. S2). The different cells were pre-incubated with 2µM TPA for 2h at 37°C before irradiation. Emission slit settings: 560-720 and 530-690 nm for **TP2Py** and **TP3Bzim**, respectively. Corresponding DIC transmission images illustrating the concomitant membrane blebbing (blue arrows) are shown below the fluorescence images.

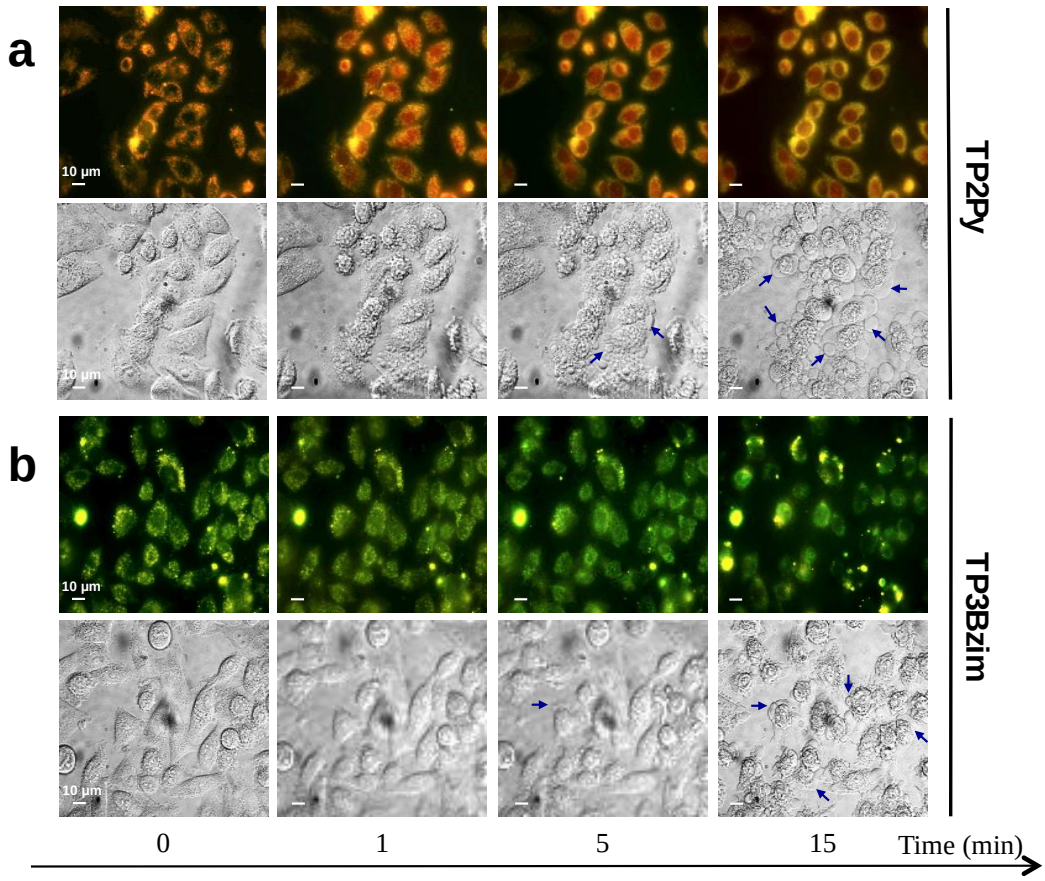

Fig. S4 Epifluorescence imaging of living HeLa cells treated with TPAs. Time-dependent re-localization of the fluorescence signal from the cytoplasm to the nucleus after light excitation of **TP2Py** (a) or **TP3Bzim** (b). Corresponding DIC transmission images illustrating the concomitant membrane blebbing (blue arrows) are shown below the fluorescence images. HeLa cells were pre-incubated with 2 µM TPA for 2h at 37°C before observation. Excitation filter, 450-490 nm; emission filter, 515-700 nm. Irradiance, 1.8 W/cm^2. The subcellular localization of TPAs in living HeLa cells, at early times of observation, was mainly cytoplasmic. The observation/excitation in the epifluorescence mode triggered a rapid relocalization of the fluorescence signal from the cytoplasm to the nucleus. This nuclear translocation began at t=1 min (corresponding to a light dose or fluence of 108 J/cm^2) and nuclear accumulation was optimal between 5 and 15 min. The formation of membrane blebs was detected in the transmission mode at t=5 min and subsequent dramatic morphological changes (nucleus and cell shrinkages) were observed at t=15 min, highly suggesting that cells underwent apoptosis, confirming confocal observations (Fig. 1 & Figs S2-S3). Similar results were obtained with living MCF7 cells (data not shown).

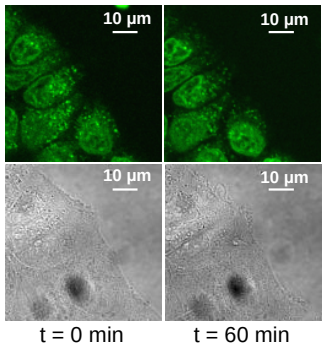

Fig. S5 Confocal fluorescence imaging of living MCF7 cells treated with 2µM of a neutral TPA, **TP3^{N}Bzim**, for 2h at 37°C before observation. Left, initial observation (t=0). Right, observation after an illumination time of 60 min (λ_{exc} = 458 nm; irradiance, 30 mW/cm^2). Top: fluorescence; bottom: DIC transmission.

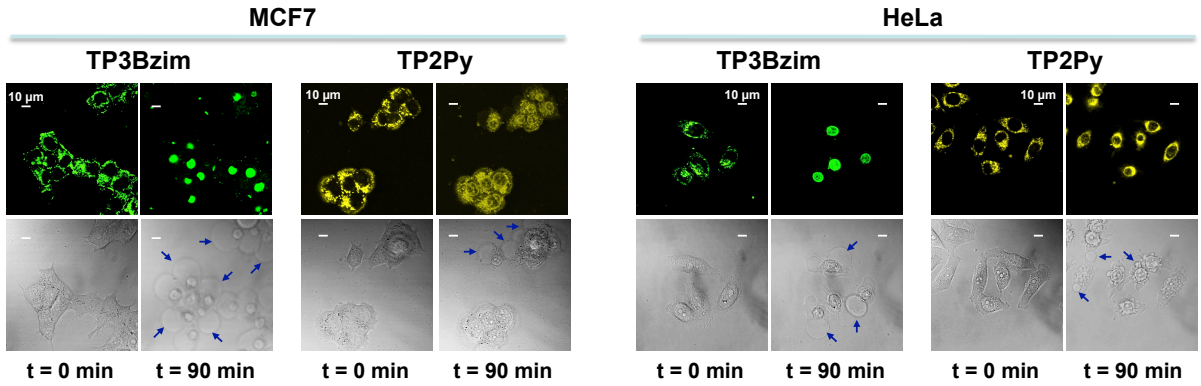

Fig. S6 Photoactivation by an external source of light is strictly required for nuclear translocation of TPAs and concomitant membrane blebbing. Confocal imaging of living MCF7 or HeLa cells pre-treated with 2µM **TP3Bzim** or **TP2Py** for 16h at 37°C before observation. Left: initial observation (t = 0) just after the 16h-incubation step showing that the initial cytoplasmic localization of TPAs was consistently observed, regardless of the incubation time, 16h or 2h (compare with Fig. 1 and Figs S2-S3). Furthermore, similar results were obtained, irrespective of the procedure used for the pre-incubation step (TPA-treated cells pre-incubated in the dark or exposed to daylight). Right: Photo-induced nuclear translocation of TPAs accompanied by the appearance of membrane blebs (blue arrows in the transmission mode) as observed at t = 90 min, showing that the 16h-incubation step did not influence the subsequent kinetics of nuclear translocation or membrane blebbing. λ_{exc} = 458 nm; irradiance, 30 mW/cm^2; emission slit settings: 560-720 nm and 530-690 nm for **TP2Py** and **TP3Bzim**, respectively.

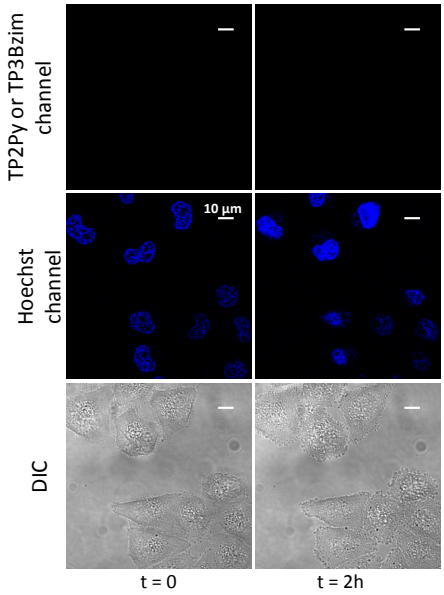

Fig. S7 Confocal fluorescence imaging of irradiated living HeLa cells in the absence of TPA. HeLa cells were incubated with Hoechst 33342 only (1µM) and exposed to visible light irradiation for 2h (λ_{exc} = 458 nm; irradiance, 30 mW/cm^2). As expected, no fluorescence of TPA was observed in the **TP2Py** channel (emission slit setting: 560-720 nm) or the **TP3Bzim** channel (emission slit setting: 530-690 nm). Hoechst channel: 406-506 nm (excitation: 750 nm). The corresponding DIC transmission images did not show any membrane blebbing in contrast to that observed in the presence of TPA (compare with Fig. 1 and Figs S2-S3).

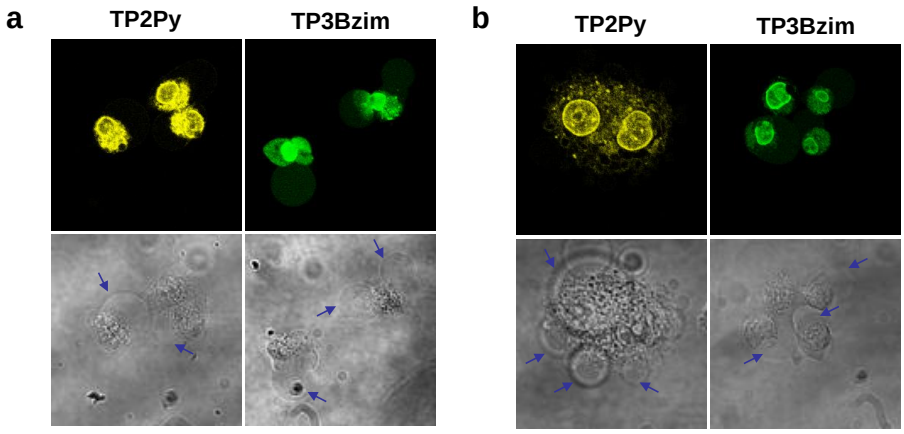

Fig. S8 Initial nuclear location of TPA after treatment with camptothecin (CPT). (a) Living HeLa cells were pre-incubated with **TP2Py** or **TP3Bzim** (2 µM) for 2h at 37°C and further incubated with CPT (20 µM) for 2h at 37°C before observation. (b) Living HeLa cells were preincubated with CPT (20 µM) for 2h at 37°C and futher incubated with **TP2Py** or **TP3Bzim** (2 µM) for 2h at 37°C before observation. Both procedures led to similar observations, i.e. an initial nuclear location of the TPA fluorescence signal (instead of the initial cytoplasmic location using TPA-treated cells in the absence of CPT), which can only be due to the proper effect of CPT since the duration of the incubation time has no effect on the initial cytoplasmic location of TPAs in the absence of any other apoptotic factor (see Figs 1, S2, S3 and S6). Excitation: 458 nm; emission slit settings: 560-720 and 530-690 nm for **TP2Py** and **TP3Bzim**, respectively. Corresponding DIC transmission images illustrating the concomitant membrane blebbing (blue arrows) are shown below the fluorescence images.

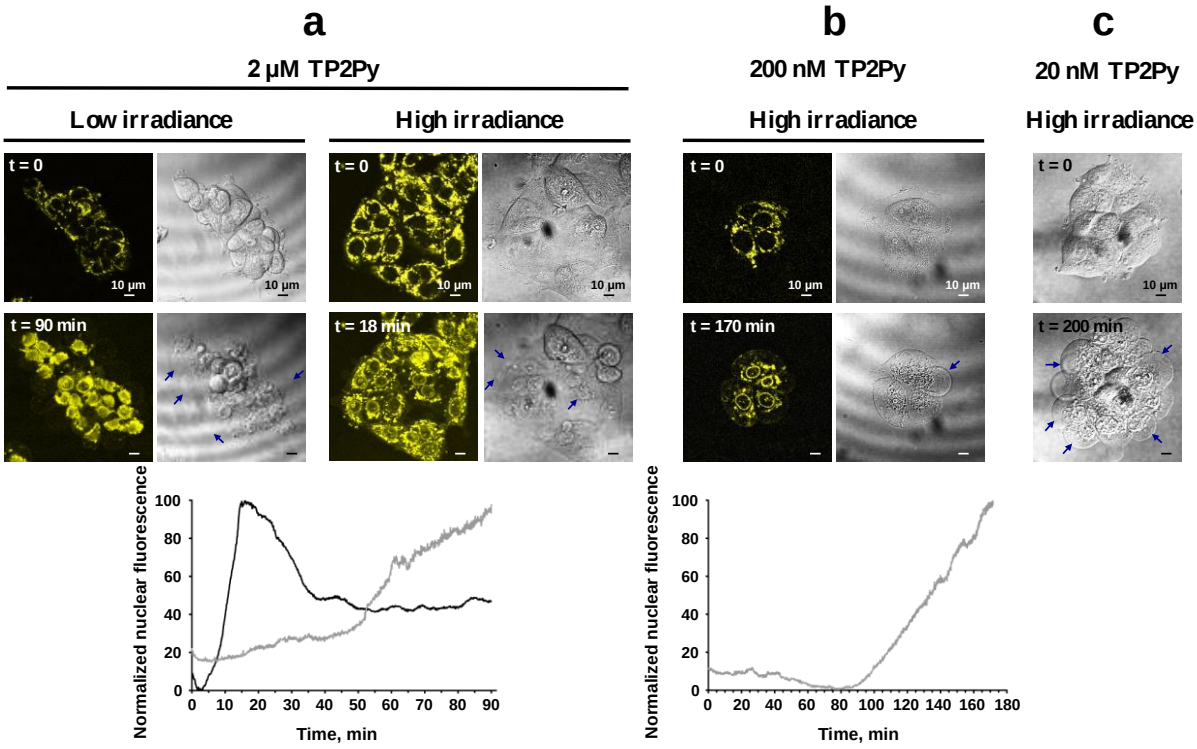

Fig. S9 Influences of the irradiance and concentration on the kinetics of nuclear re-localization of TPAs. (a) Living MCF7 cells were treated with 2 µM **TP2Py** for 2h at 37°C and observed using confocal microscopy at two irradiances (left, 5.6 mW/cm^2; right, 30 mW/cm^2). Top: fluorescence and DIC transmission images at t = 0, corresponding to the initial observation (beginning of the excitation). Bottom: fluorescence and transmission images at a time corresponding to the first peak of nuclear accumulation (as shown in Fig. 1c). The kinetics of nuclear accumulation of **TP2Py** are shown below for two irradiance values: 5.6 mW/cm^2 (grey) or 30 mW/cm^2 (black). λ_{exc} = 458 nm; emission slit setting: 560-720 nm. (b-c) Same conditions as above except that the **TP2Py** concentration was decreased: 200 and 20 nM, respectively (irradiance, 30 mW/cm^2). The fluorescence image corresponding to [TP2Py] = 20 nM is not shown for detection reasons, although membrane blebbing (blue arrows) is clearly visible in the transmission mode.

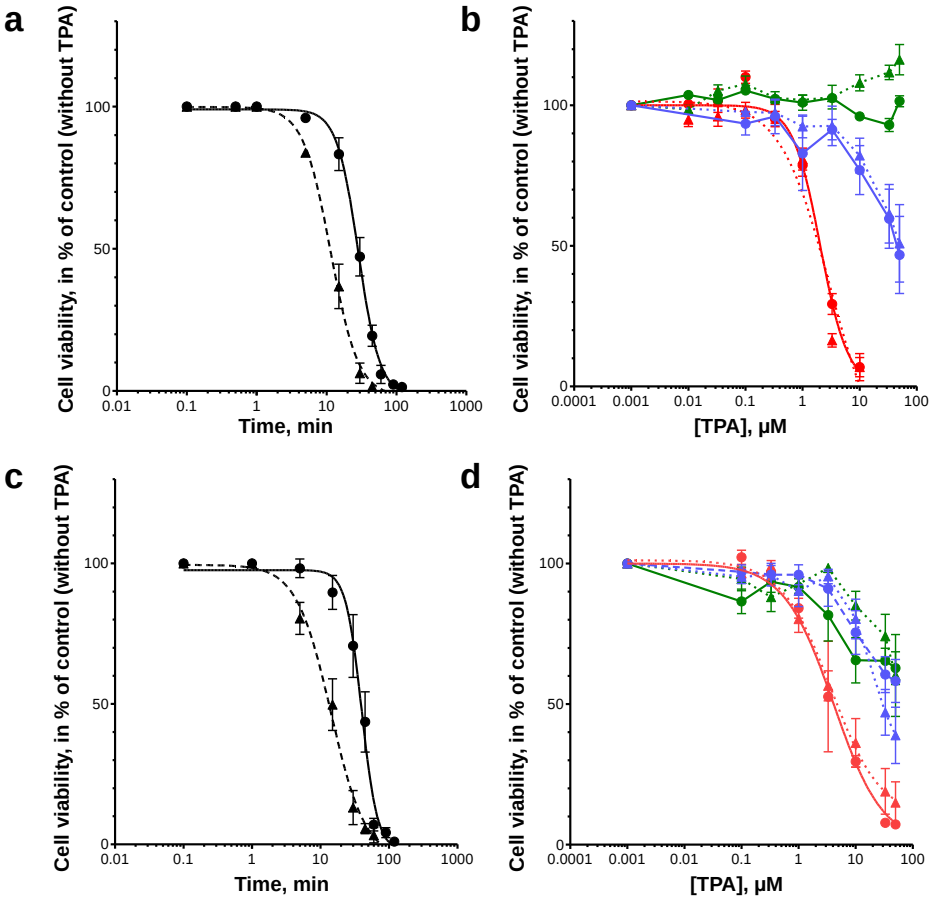

Fig. S10 Photocytotoxicity of TPAs in HeLa (a & b) and MCF7 (c & d) cells. Cell viability (in % of untreated cells) as a function of illumination time (for a given TPA concentration) (a & c) or as a function of TPA concentration (for a given illumination time) (b & d).

(a & c) Cells were treated with 2 µM **TP2Py** (black circles) or **TP3Bzim** (black triangles) for 2h at 37°C and further illuminated at 452 nm for varying times. The times of light exposure lethal for 50% of the cells (LT_{50}) were 15 and 30 min for TP3Bzim and TP2Py, respectively, in HeLa cells (corresponding light dose values: 15.3 and 30.6 J/cm^2, respectively). LT_{50} were 15 and 40 min for TP3Bzim and TP2Py, respectively, in MCF7 cells (corresponding light dose values: 15.3 and 40.8 J/cm^2, respectively).

(b & d) Cells were treated with varying concentrations of TPA (circles: **TP2Py**; triangles: **TP3Bzim**) and subjected to light irradiation at 452 nm during a time period corresponding to LT_{50} (red curves) as determined in panels a & c, subjected to daylight illumination for 30 min (blue curves) or protected from light (green curves).

The source of excitation was a Mercury lamp (130 W, wavelength 380-600 nm) coupled to a filter (λ = 452 ± 45nm); irradiance, 17 mW/cm^2.

In HeLa cells, LT_{50} values (time of light exposure lethal for 50% of the cells) were 30 and 15 min for **TP2Py** and **TP3Bzim**, respectively (corresponding to light dose values of 30.6 and 15.3 J/cm^2, respectively) indicating that **TB3Bzim** was a slightly more potent cell death inducer than **TP2Py** under this illumination condition (LT_{50} = 40 and 15 min for **TP2Py** and **TP3Bzim** in MCF7 cells, corresponding to light dose values of 40.8 and 15.3 J/cm^2, respectively). For HeLa cells, the dark toxicity was negligible up to 50 µM TPA. Moreover, at the TC_{50} value (2 µM; concentration of TPA leading to 50% lethality under light irradiation at 452 nm), no toxicity was detected with daylight exposure. However, a slight daylight toxicity was observed with TC_{50} values around 50 µM for both TPAs. Accordingly, we observed both membrane blebbing and nuclear translocation of TPAs after 2-hour incubation of 30-50 µM TPA with HeLa cells exposed to daylight (data not shown). General characteristics were similar using MCF7 cells. Although moderate, the dark toxicity of TPAs was higher in MCF7 than in HeLa cells (TC_{50} = 60-65 µM). Taken together, these results confirm that TPAs induce light-dependent cell death in the low micromolar range, in agreement with imaging experiments; dark or daylight toxicities are negligible or moderate below 30 µM.

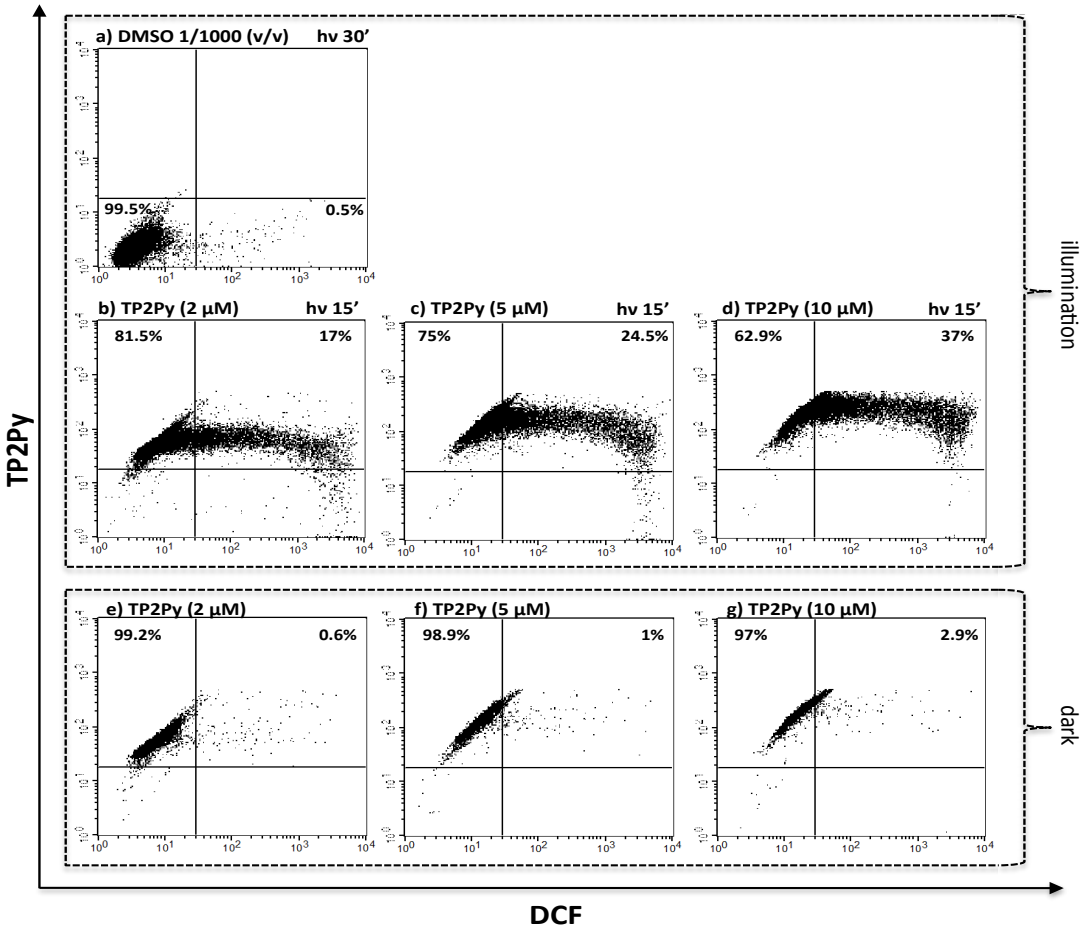

Fig. S11 Concentration dependence of ROS production upon photoactivation of TPAs. Living HeLa cells were plated (in 12-well plates) and treated with varying concentrations of **TP2Py**: 2 µM (*b* & *e*), 5 µM (*c* & *f*) or 10 µM (*d* & *g*). Before addition of 20 µM H_2DCF-DA and flow cytometric analysis, the cells were subjected (*b*-*d*) or not (*e*-*g*) to light exposure. Negative control (cells treated with DMSO alone and subjected to light illumination) is shown in *a*. The source of excitation was a Mercury lamp (130 W; 380-600 nm) with an excitation filter centered at 452 nm (± 45 nm); irradiance, 17 mW/cm^2. The horizontal and vertical axes correspond to fluorescence intensities of DCF and **TP2Py**, respectively.

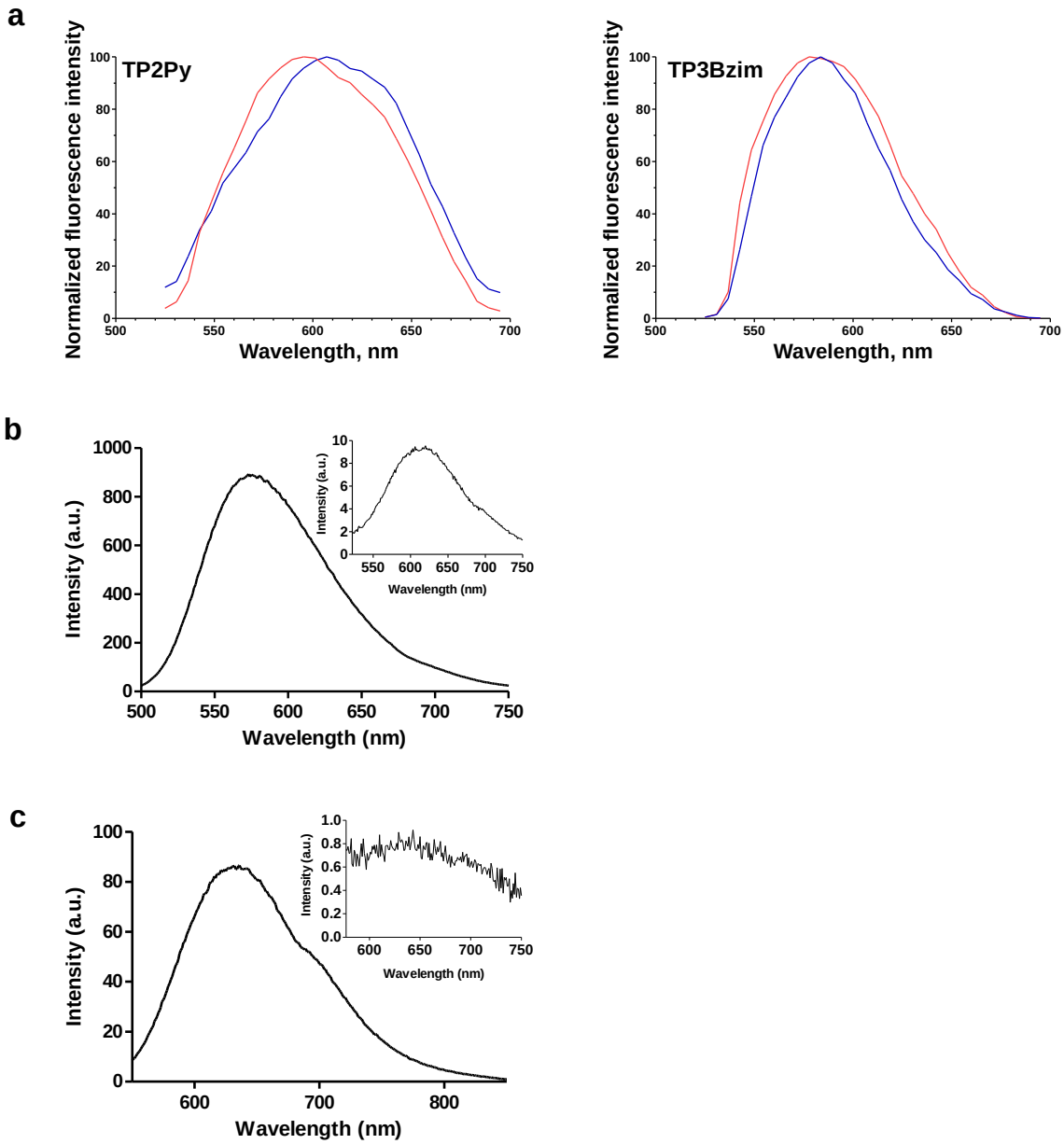

Fig. S12 Fluorescence emission spectra of **TP2Py** (left) and **TP3Bzim** (right) in distinct ROIs (region of interest; see Fig. 1c) corresponding to the nucleus (blue) or the cytoplasm (red) of MCF7 cells (a). The emission spectrum of **TP3Bzim** in the cell context was unchanged (maximum about 580 nm), regardless of its localization (nuclear or cytoplasmic). This emission is close to the characteristic maximum found *in vitro* for the bound form of **TP3Bzim**, 572 nm (for example, when bound to DNA; panel b: λ_{exc} = 457 nm) and significantly different from the maximum characterizing the free compound, 620 nm (inset of panel b: λ_{exc} = 433 nm). Such a conclusion is not direct in the case of **TP2Py** as the emission maxima are similar *in vitro* for bound (panel c: λ_{exc} = 506 nm) and for free (inset of panel c: λ_{exc} = 473 nm) forms, 637 and 633 nm, respectively; the fluorescence emission spectra of **TP2Py** in nuclei and in cytoplasm were compatible with these emission spectra (the maximum of emission was centered at 615 nm in nuclei and slightly blue-shifted in the cytoplasm: 602 nm).

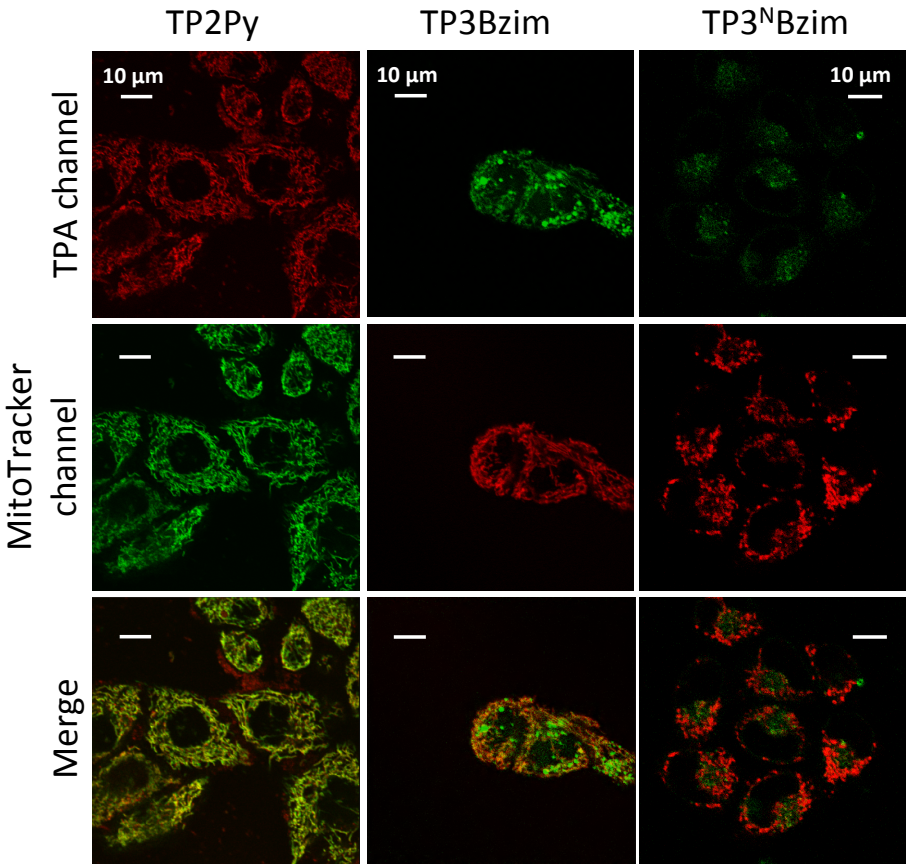

Fig. S13 Colocalization between cationic TPAs and mitochondria in HeLa cells before light-induced nuclear translocation. Mitochondria staining was performed with a green tracker of mitochondria (MitoTracker® Green FM; λ_{exc} = 488 nm / emission: 500-550 nm) for **TP2Py**, and a red tracker (MitoTracker® Red FM; λ_{exc} = 633 nm / emission: 650-700 nm) for both **TP3Bzim** and **TP3^{N}Bzim**. TPA channel: λ_{exc} = 458 nm / emission slit settings: 560-720 and 530-690 nm for **TP2Py** and **TP3Bzim**/**TP3^{N}Bzim**, respectively. Top: imaging channel of TPA; middle: imaging channel of MitoTracker; bottom: corresponding merged images. Yellow to orange areas indicate colocalization of TPA/mitochondria. Only the two cationic compounds, **TP2Py** and **TP3Bzim**, but not the neutral **TP3^{N}Bzim** compound, display a significant colocalization signal with mitochondria.

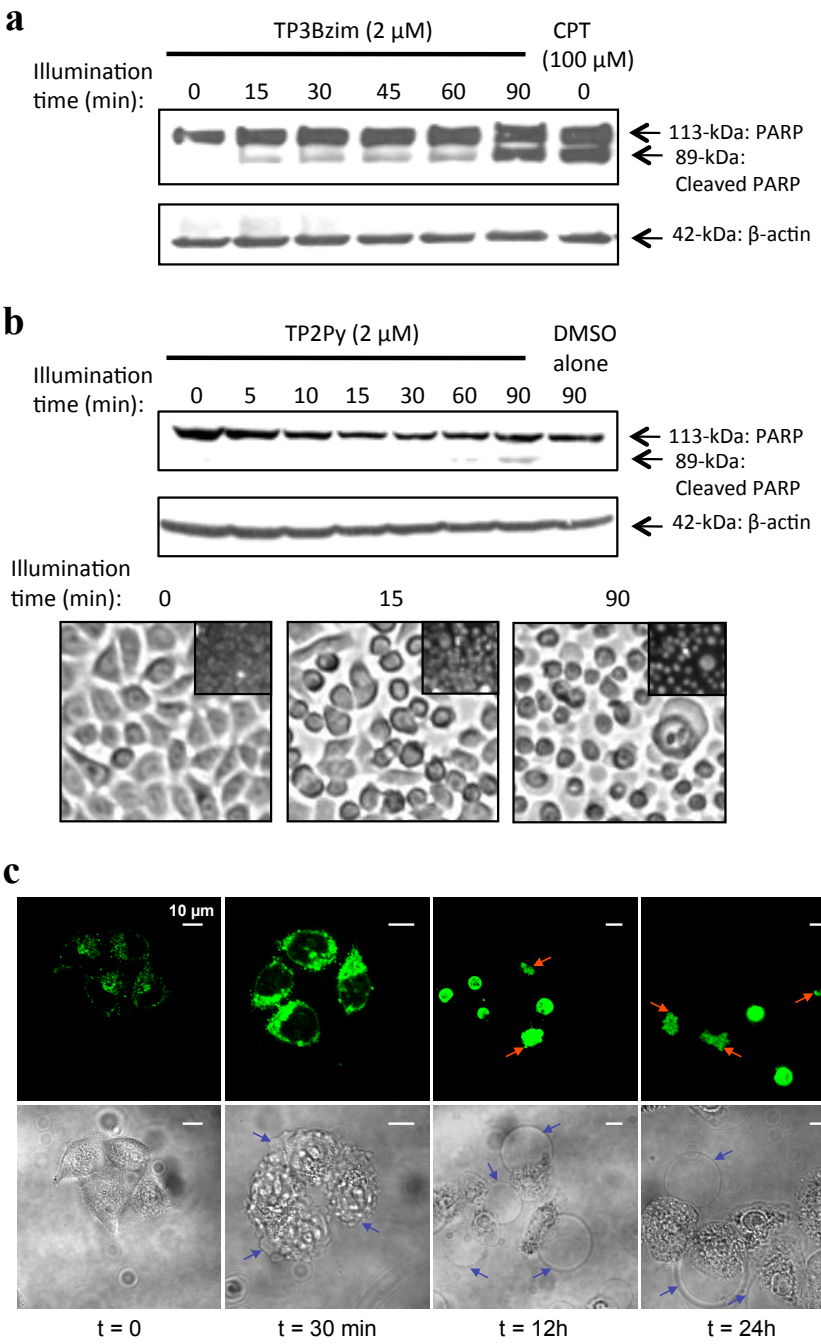

Fig. S14 TPAs induce cell apoptosis upon light illumination as revealed by the PARP-1 cleavage assay. HeLa cells were plated (in 12 well-plates) and treated with 2 µM **TP3Bzim** (a) or **TP2Py** (b). After 2h incubation at 37°C, the cells were illuminated at 452 nm during varying times (up to 90 min). The source of excitation was a Mercury lamp (130 W, wavelength 380-600 nm) coupled to a filter (λ = 452 ± 45 nm); irradiance, 17 mW/cm^2. At the end of illumination, the cells were lysed and the cleavage of PARP-1 was detected by Western blot analysis (typically 40 µg proteins/lane), using an anti-PARP-1 antibody that recognizes both the entire (113-kDa) and cleaved PARP-1 (89-kDa). The immunodetection of β-actin (42-kDa) was used as an internal loading control. Camptothecin (CPT; 100 µM) and DMSO (1% (v/v)) under illumination condition were used as positive and negative controls, respectively. Panel b, bottom: transmission images showing the morphology of HeLa cells after incubation with **TP2Py** (2 µM) without any light illumination treatment (left) or with a light illumination treatment of 15 min (middle) or 90 min (right). Insets: epifluorescence images. (c) Confocal fluorescence imaging of **TP3Bzim**-treated HeLa cells. HeLa cells were pre-incubated with 2 µM **TP3Bzim** for 2h at 37°C and observed before (t = 0) and after 30-min light irradiation (t = 30 min) using the Mercury lamp as described above. **TP3Bzim**-treated HeLa cells were then kept in an atmosphere of 5% CO_2 at 37°C and observed at 12h and 24h post-irradiation. The red arrows indicate events of nucleus disintegration. Corresponding DIC transmission images illustrating membrane blebbing (blue arrows) are shown below the fluorescence images.

The cleavage of PARP-1 was assessed by Western blot using an anti-PARP-1 antibody that recognizes both the entire and cleaved PARP-1 (Fig. S14a-b). HeLa cells were pre-treated with either **TP3Bzim** or **TP2Py** (2-h incubation) and further illuminated at 452 nm for various illumination times (up to 90 min). PARP-1 cleavage was detected in the presence of both compounds and its intensity was dependent on the illumination time; no cleavage was observed with untreated cells (DMSO only) after 90-min illumination. However, a differential efficiency of **TP2Py**/**TP3Bzim** was observed: **TP3Bzim** led to a significant PARP-1 cleavage after 15-min illumination (the cleavage efficiency after 90-min illumination was comparable with the one obtained under CPT treatment) whereas **TP2Py**-dependent PARP-1 cleavage was observed after a significantly longer illumination time (typically 60 min). Such a quantitative differential effect of the two compounds on PARP-1 cleavage is in accordance with photocytotoxicity results, due to the illumination condition (452 ± 45 nm) which is more favourable to **TP3Bzim** (λ_{abs} = 457 nm) than **TP2Py** (λ_{abs} = 506 nm). The detection of PARP-1 cleaved products was accompanied by alteration of the cell morphology induced by the combination of TPA and light illumination. Indeed, after a 2-h pre-incubation with **TP2Py** but without any light illumination, HeLa cells displayed a normal morphology in transmission images. After 15-min illumination, a few cells were detached, whereas all the cells displayed morphological alterations such as cell shrinkage typically observed during apoptosis, after 90-min illumination. More dramatic morphological change such as nucleus disintegration was observed from t = 12h (and confirmed at t = 24h) after visible light irradiation (30-min illumination; Fig. S14c).

Movie S1 Video movies (17s each; acceleration x900: 1s <-> 15min) showing the photo-induced cell death of MCF7 cells in the presence of **TP3Bzim**. (a) Confocal images. (b) DIC transmission images. (c) Merged images. Experimental conditions are similar to those mentioned in the legend of Fig. 1.

SUPPLEMENTARY MATERIALS AND METHODS

Synthesis and reagents.

Synthesis of TP2Py (4,4'-bis[(E)-2-(pyridin-4-yl)vinyl]triphenylamine bis-methiodide) and TP3Bzim (2,2',2''-((1E,1'E,1''E)-(nitrilotris(benzene-4,1-diyl))tris-(ethene-2,1-diyl))tris(1,3-dimethyl-1H-benzo[d]imidazol-3-ium)iodide) were performed as previously described.[1]

Synthesis of TP3^{N}Bzim (tris[4-(1H-benzimidazol-2-ylethenyl)phenyl]amine). To a suspension of 4,4'-trisformyltriphenylamine (120 mg, 1 equiv.) and phosphonium salt (625 mg, 4 equiv.) in 15 ml of dry methanol was added sodium methylate (15 equiv.) in methanol. The mixture was stirred for 24h in the dark and then concentrated to dryness. The residue was diluted in dichloromethane. Pentane was added until precipitation of a yellow solid. The precipitate was filtered under reduced pressure, washed with pentane and dried under vacuum to afford 165 mg (86%) of the expected compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.07 (s, 3H), 7.79-7.59 (m, 9H), 7.59-7.46 (m, 6H), 7.27-7.05 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.6, 150.3, 147.1, 137.2, 135.3, 130.4, 128.0, 123.8, 122.6, 113.8, 113.5; MS (ESI+) m/z 672.3.

Reagents. Tert-butyl hydroperoxide (TBHP), camptothecin (CPT), N-acetyl cysteine (NAC), cyclosporine A (CsA) and 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA) were from Sigma-Aldrich. Phenyl-methylsulfonyl fluoride (PMSF) and sodium orthovanadate were from Thermo Scientifc Pierce and EMD Millipore, respectively. Hoechst 33342 was from Molecular probes.

Cell lines. Five human cancer cell lines were tested throughout this study: HeLa (cervical cancer), MCF7 (breast cancer), LNCaP (prostate adenocarcinoma), HepG2 (hepatocellular liver carcinoma) and Jurkat cells (T-lymphoid leukemia cell line).

Spectroscopic characterization and instrument set-up for fluorescence imaging. Fluorescence emission spectra of TPAs were measured at 25°C on a Cary-Eclipse spectrofluorimeter (Varian, CA, USA) equipped with a thermostated cell holder, using solutions (70 µl) placed in microcuvettes (pathlength, 0.3 cm). The PMT (photomultiplier tube) voltage was 630 V. The TPA concentration was 5 µM in Tris buffer (10 mM Tris-HCl, pH 7.2, 100 mM KCl) in the presence or absence of 10 µM 21-mer double-stranded DNA (U5A/B); U5A (5'-cct gct agg gat ttt cct gcc-3') and its complementary strand were annealed as previously described.[2]

Epifluorescence and confocal images were obtained using a SP2 confocal microscope (Leica MicroSystem) equipped with an oil immersion x63 objective (numerical aperture, 1.32) and an incubation chamber (37°C, CO_2 5%). Excitation and emission filters for epifluorescence were 450-490 nm and 515-700 nm, respectively. A continuous laser line (458 nm) was used for excitation of TPAs in the confocal mode (emission slit settings are specified in figure legends). The irradiances were calculated by measuring the power at the exit of the objective using a Vega power meter (Ophir). The power measurement for confocal experiments was performed in the x-y scan mode (image size, 512x512 pixels; field of view, 140x140 µm; scanning frequency, 800 Hz). The illuminated surface area was 10^{-3} cm^2 in the epifluorescence mode. Fluorescence emission spectra of TPAs in the cellular context were monitored using the lambda-scan mode of the confocal microscope. Two-photon excitation of Hoechst 33342 was performed using a 80-MHz mode-locked Mai-Tai® Ti:Sapphire tunable laser (720-920 nm, 100 fs laser pulse; Spectra Physics, Mountain View, California) tuned to 750 nm.

Colocalization experiments with mitochondria. HeLa cells were pre-treated with TPA for 2h at 37°C. The cells were then washed with PBS buffer (phosphate-buffered saline, pH 7.5, Gibco®) and further incubated with 20 nM MitoTracker® (Invitrogen) for 30 min. For TP2Py (red emission) and TP3Bzim/TP3^{N}Bzim (green emission), MitoTracker® Green FM and MitoTracker® Red FM were used, respectively. Concentrations of TPAs were 2 µM (TP2Py & TP3Bzim) or 10 µM (TP3^{N}Bzim).

Photocytotoxicity experiments. HeLa or MCF7 cells were plated in 96-well plates (5.10^3 cells/well), pre-incubated with TPAs for 2h in the dark at 37°C and subjected to light exposure. The excitation source was a Mercury lamp (130 W; 380-600 nm) equipped with an excitation filter centered at 452 nm (± 45 nm) (irradiance, 17 mW/cm^2, measured using a Vega power meter on a total illuminated surface of 3.8 cm^2 (equiv. 4 wells)). TPA concentrations and illumination times are indicated in the corresponding figure legend. After further incubation of treated cells for 24h at 37°C, cell viability was determined by a colorimetric MTT assay, according to a standard protocol.[3] The absorbance at 570 nm was measured using a Victor^{TM} X5 microplate reader (Perkin Elmer).

PARP-1 detection by western blotting analysis. HeLa cells were plated in 12-well plates (2.10^5 cells/well) and pre-incubated with 2 µM TPA for 2h in the dark at 37°C. After varying illumination times at 17 mW/cm^2 (Mercury lamp (130 W; 380-600 nm) + excitation filter centered at 452 nm (± 45 nm); total illuminated surface: 3.8 cm^2 (equiv. 1 well)), cells were collected by scraping, washed once in ice-cold PBS, treated with lysis buffer (20 mM Tris, pH 8.0, 137 mM NaCl, 10% glycerol (v/v), 1% Nonidet P-40 (v/v)) supplemented with 1mM PMSF, 1 mM sodium orthovanadate and anti-protease cocktail (Complete, Boehringer Mannheim, Germany)) for 20 min on ice and further centrifuged at 10,000 g for 10 min. Protein concentration was determined using the bicinconinic acid method (micro BCA, Pierce, USA). Detergent soluble proteins (40 µg) were separated by SDS-PAGE (4-12% polyacrylamide gradient gel) under reducing conditions followed by Western blotting. Membranes were first incubated overnight at 4°C with the rabbit primary polyclonal antibody anti-PARP-1 (#9542, Cell Signaling Technology). Membranes were then probed with horseradish peroxidase-labelled anti-rabbit IgG (dilution 1/2000; Dakocytomation). Detection was based on a chemiluminescence detection system (GE Healthcare, Orsay, France).

Fluorescence detection of ROS generation. ROS detection was performed using H_2DCF-DA with TP2Py only for spectral reasons.

For flow cytometric analysis, HeLa cells were plated in 12-well plates (2.10^5 cells/well) and pre-incubated with 2 µM TP2Py for 2h at 37°C. After light exposure for 30 min using a Mercury lamp (130 W; 380-600 nm; excitation filter centered at 452 nm (± 45 nm); irradiance: 17 mW/cm^2; total illuminated surface: 3.8 cm^2 (equiv. 1 well)), the cells were treated with H_2DCF-DA according to the manufacturer's instructions (Sigma). After H_2DCF-DA treatment, the cells were trypsinized and centrifuged at 1,500 rpm for 5 min. The cell pellets were resuspended in DMEM (Gibco®) supplemented with 10% FBS (Gibco®) and analysed by FACSCalibur flow cytometer (Becton-Dickinson). Fluorescence signals of DCF and TP2Py were measured by using FL1 and FL2 channels, respectively. For the experiment in the presence of NAC, the protocol was the same except that the cells were first pre-incubated with 3 mM NAC for 12h at 37°C before the addition of TP2Py. For the experiment in the presence of CsA, the compound was added to the cells (final concentration of 10 µM) 90 min after the addition of TP2Py and 30 min before light illumination. These concentrations of NAC and CsA were chosen because they did not induce significant cell toxicity *per se*, as measured by MTT assay: NAC was not toxic up to 5 mM (100% viability) while NAC displayed significant toxicity beyond 5 mM (45% viability at 10 mM) (not shown). CsA was not or slightly toxic up to 18 µM (>90% viability; 80% viability at 25 µM) (not shown). Three negative control experiments were performed: DMSO alone (1/1000 (v/v)) with or without light illumination, or 2 µM TP2Py without light illumination. One positive control experiment was performed using cells incubated with the oxidative stress inducer TBHP (100 µM) for 3h at 37°C (without light illumination).

Direct ROS detection in living MCF7 cells was performed using confocal fluorescence imaging. TPA-treated cells were incubated with H_2DCF-DA as explained in the corresponding figure legend. A continuous laser line (488 nm) was used for excitation of DCF (emission slit setting: 500-530 nm).

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