SUPPORTING INFORMATION

Improved Photodynamic Effect through Encapsulation of Two Photosensitizers in Lipid Nanocapsules

Alexandre Barras,†[1*] Nadia Skandrani,†[1,2] Mariano Gonzalez Pisfil,[3] Solomiya Paryzhak,[4] Tetiana Dumych,[4] Aurélien Haustrate,[5] Laurent Héliot,[3] Tijani Gharbi,[2,6] Hatem Boulahdour,[2,6] V'yacheslav Lehen'kyi,[5] Rostyslav Bilyy,[4] Sabine Szunerits,[1] Gabriel Bidaux,[3] and Rabah Boukherroub,[1*]

[1]*Univ. Lille, CNRS, Centrale Lille, ISEN, Univ. Valenciennes, UMR 8520 - IEMN, F-59000 Lille, France*

[2]*Laboratoire de Nanomédecine, Imagerie et Thérapeutique, Université de Franche-Comté, 16 Route de Gray, 25030 Besançon, France*

[3]*Laboratoire de Physique des Lasers, Atomes and Molécules, Equipe Biophotonique Cellulaire Fonctionnelle, Parc scientifique de la Haute Borne, Villeneuve d'Ascq, France*

[4]*Danylo Halytsky Lviv National Medical University, 79010 Lviv, Ukraine*

[5]*Univ. Lille, Inserm, U1003 – PHYCEL – Physiologie Cellulaire, LABEX ICST, F-59000 Lille, France*

[6]*CHRU Jean Minjoz, 3 Bd Alexandre Fleming, 25030 Besançon, France*

*To whom correspondence should be addressed: alexandre.barras@univ-lille1.fr; rabah.boukherroub@univ-lille1.fr; Tel: +333 62 53 17 24; Fax: +333 62 53 17 01. †These authors contributed equally to this work.

Preparation and physical characterization of PS-loaded LNC25

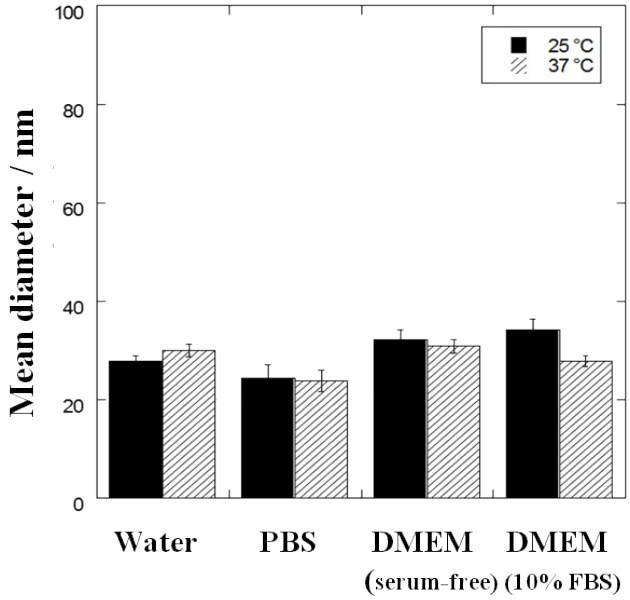

Figure S1: Mean diameter of LNC25 in different media after 1 h incubation.

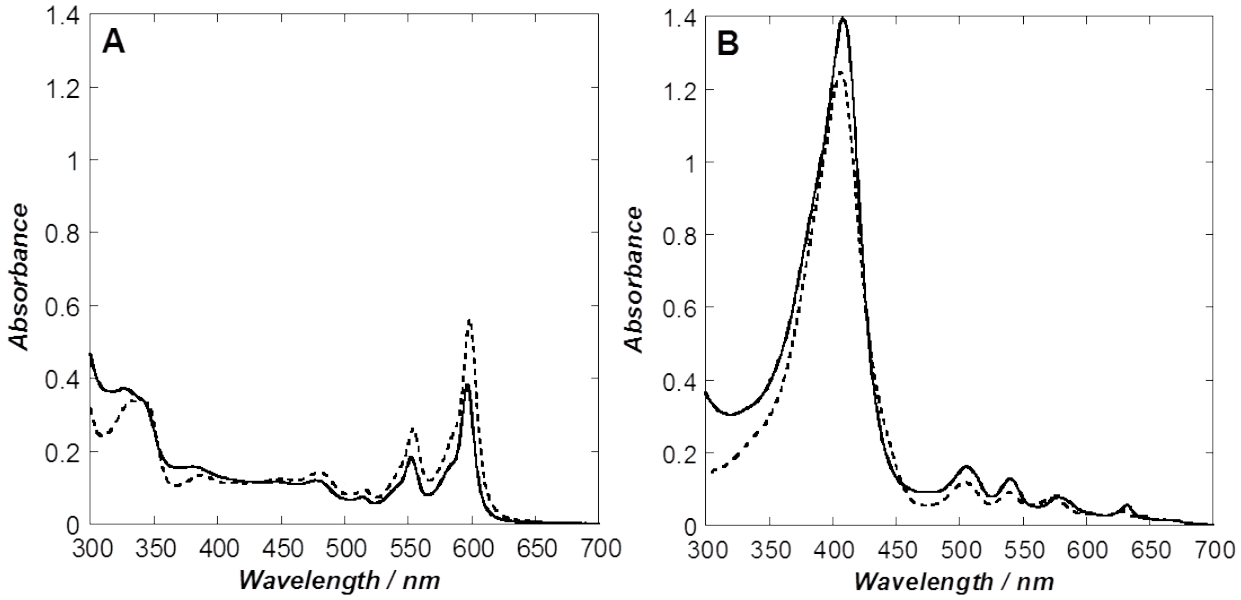

Figure S2. Absorption spectra (300 - 700 nm) of Hy dissolved in DMSO (*dotted line*) and Hy-loaded LNC25 (*continuous line*) in water both at 10 µM **(A)**; PpIX dissolved in DMSO (*dotted line*) and PpIX-loaded LNC25 (*continuous line*) in water both at 10 µM **(B)**.

Fluorescence properties of PS-loaded LNC25

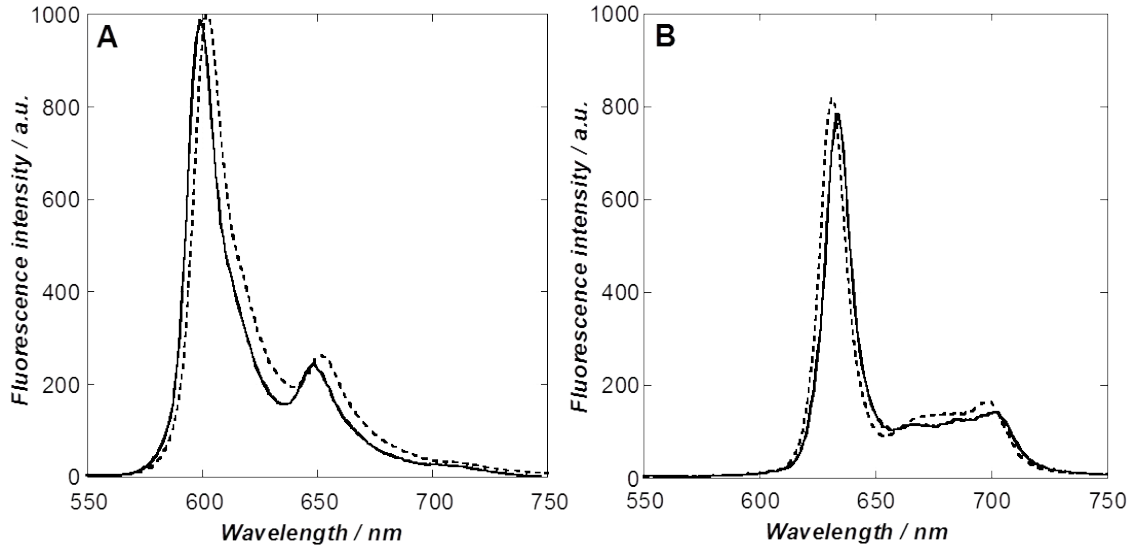

Figure S3. Fluorescence emission spectra (550-750 nm) of Hy dissolved in DMSO (*dotted line*) and Hy-loaded LNC25 (*continuous line*) in water both at 2.5 µM **(A)**; PpIX dissolved in DMSO (*dotted line*) and PpIX-loaded LNC25 (*continuous line*) in water both at 2.5 µM **(B)**. The emission spectra are recorded using an excitation wavelength λ_{ex}=330 nm **(A)** and 410 nm **(B)**.

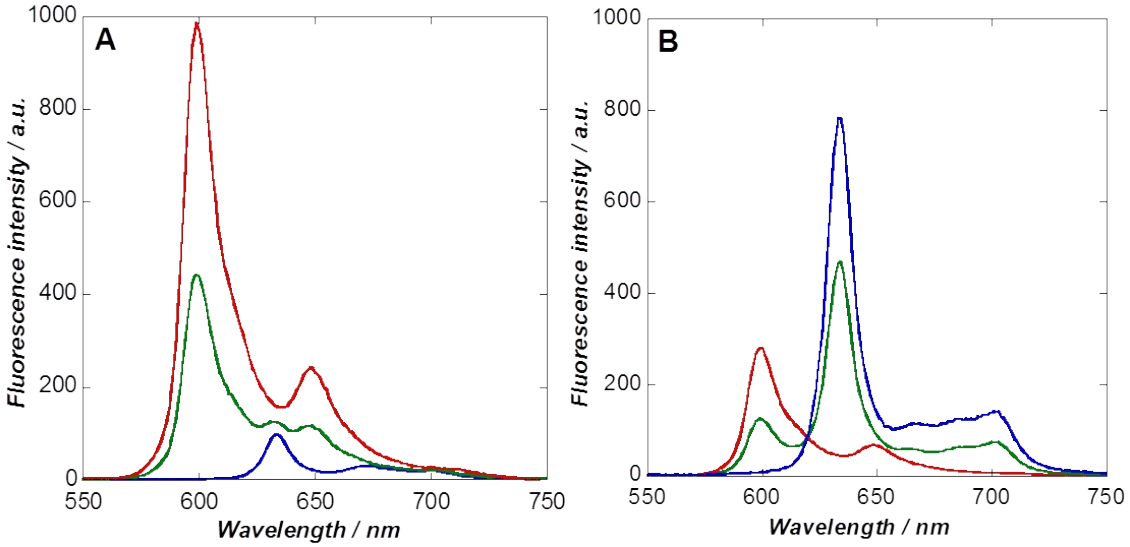

Figure S4: Fluorescence emission spectra (550-750 nm) of Hy-loaded LNC25 (*red line*), PpIX-loaded LNC25 (*blue line*) and PpIX-Hy-loaded LNC25 (*green line*) at 2.5 µM. The emission spectra are recorded using an excitation wavelength λ_{ex}=330 nm **(A)** and 410 nm **(B)**.

***In vitro* phototoxicity**

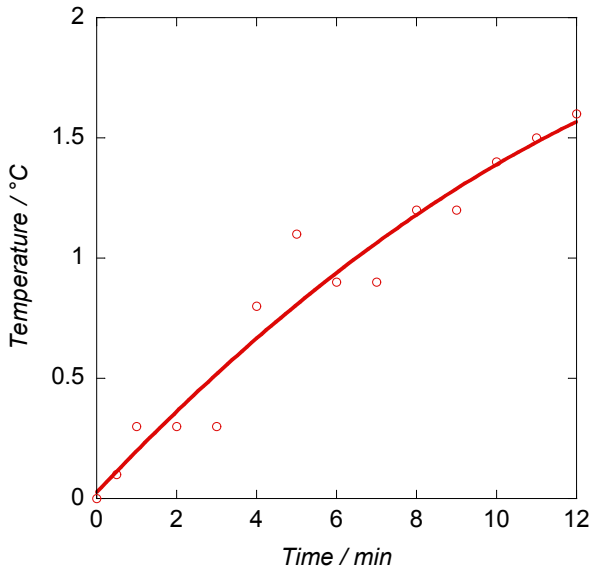

Figure S5: Temperature increase of serum-free DMEM during PDT treatment with visible light (λ>400 nm, 10 mW).

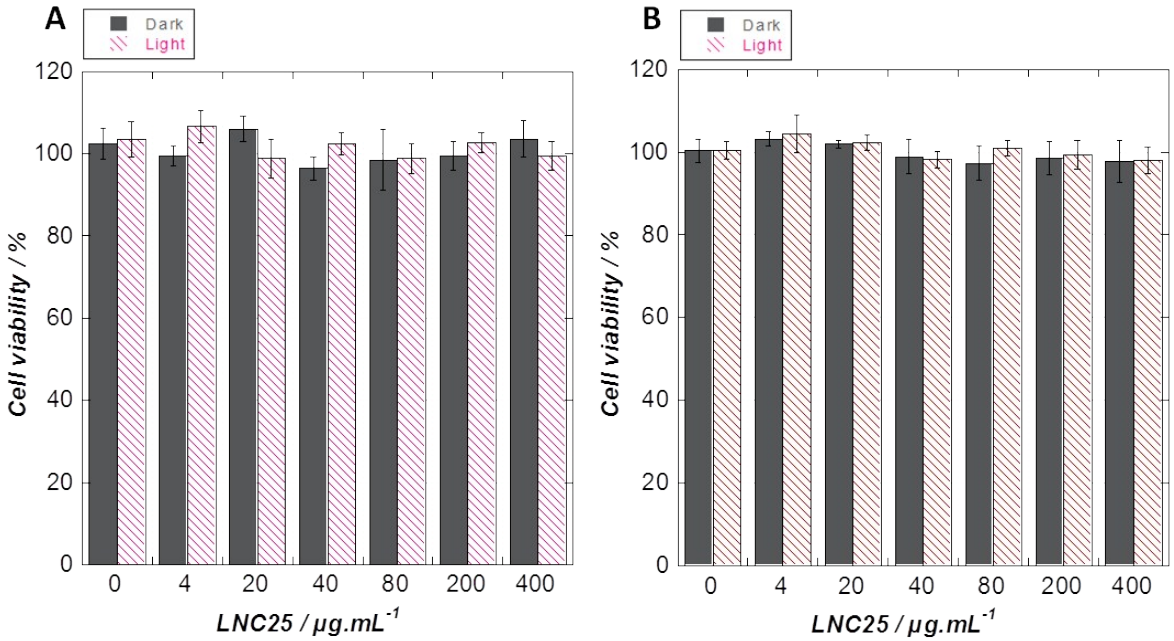

Figure S6: *In vitro* phototoxicity of blank LNC25. MTT assay data for blank LNC25 at different concentrations (incubation time 8 h) in the dark or upon visible light irradiation (12 min at 10 mW) using HeLa **(A)** and MDA-MB-231 **(B)** cell lines.

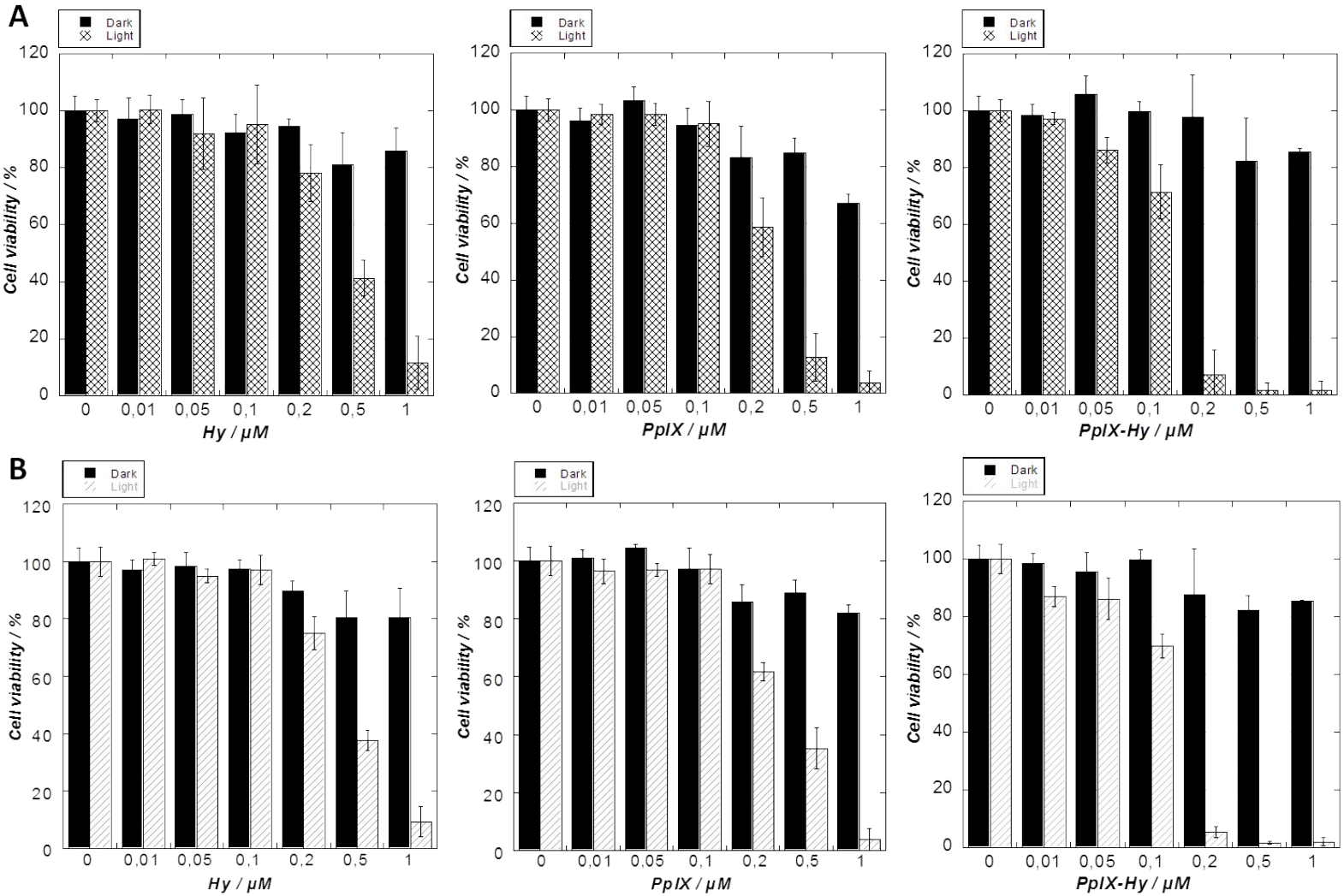

Figure S7: *In vitro* phototoxicity of free PS. MTT assay data for free hypericin (Hy), free protoporphyrin IX (PpIX) and 50/50 molar ratio of free Hy/PpIX at different concentrations (incubation time 8 h) in the dark or upon visible light irradiation (12 min at 10 mW) using HeLa **(A)** and MDA-MB-231 **(B)** cell lines.

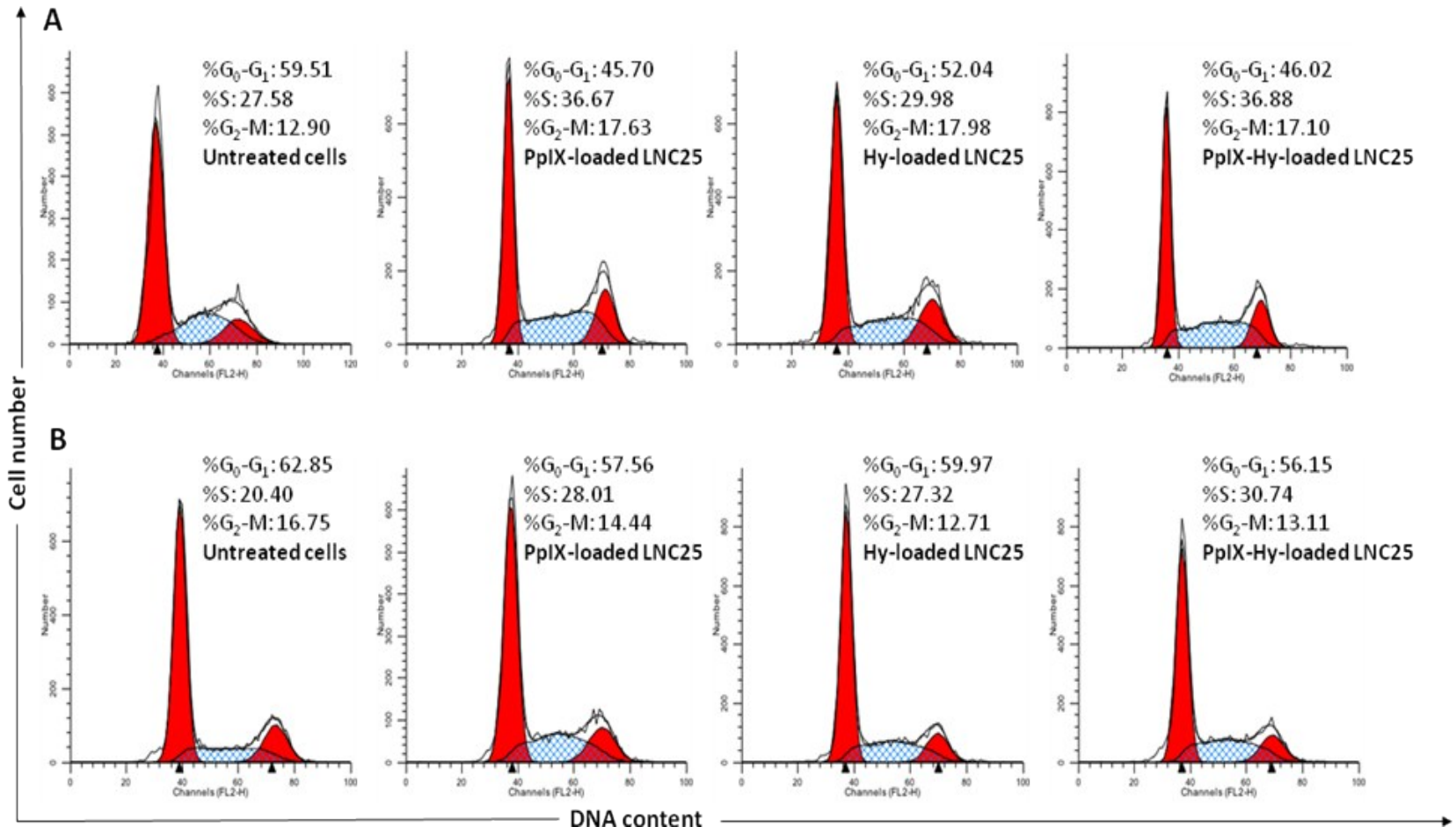

Figure S8. DNA flow cytometric analysis. The cells **(A)** MDA-MB-231 and **(B)** HeLa are treated with PS-loaded LNC25 at 0.5 μM and irradiated with visible light (10 mW) for 12 min. After fixation and staining with PI, the cells are analysed by flow cytometry. The percentage of cells in G_0-G_1, S and G_2-M are calculated using MODFIT computer software and are represented within the histograms. Statistical difference from the untreated controls: $^{*}p < 0.05$; $^{**}p < 0.01$.

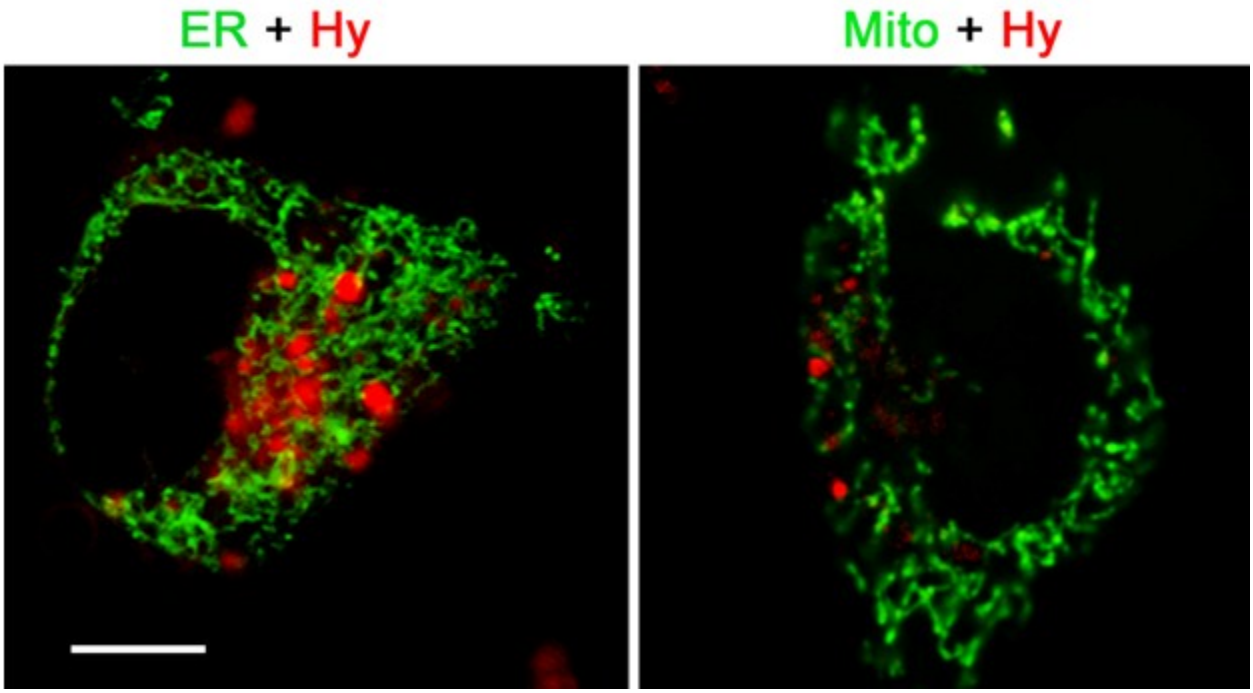

Figure S9. Intracellular localization of hypericin in HeLa cells. Cells were transfected with ER-target Ca^{2+} biosensor, D1ER (left panel), or Mitochondria-targeted Ca^{2+} biosensor, 4mtD3cpv (right panel) for 2 days prior to their treatment with Hy-loaded LNC25 (0.5 μM) for 2 h. Fluorescence imaging (512×512 pixels) was performed with a SP5 LSM (Leica Microsystems). Objective: ×60. Scale bar: 5 μm.

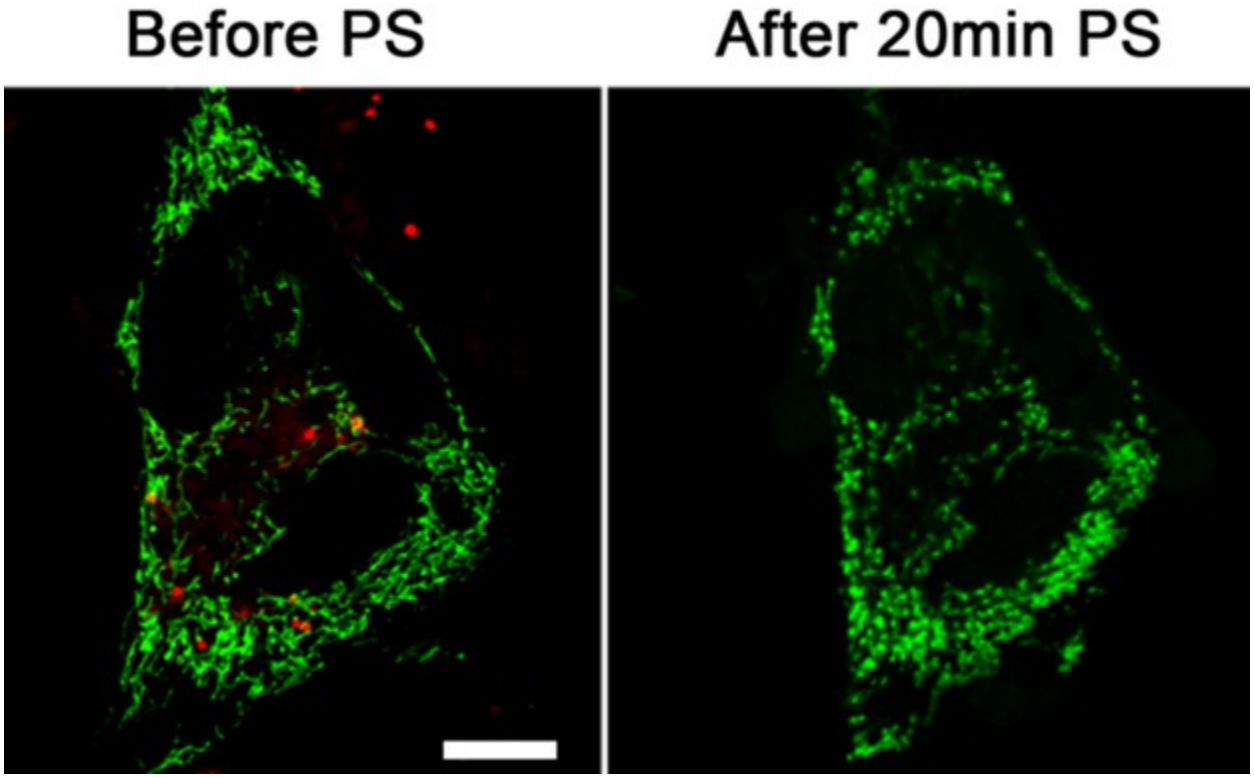

Figure S10. Fragmentation of the mitochondrial network induced by the photo-stimulation of PpIX-preloaded HeLa cells. Cells were transfected with Mitochondria-target Ca^{2+} biosensor, 4mtD3cpv for 2 days prior to their treatment with PpIX-loaded LNC25 for 2 h. Fluorescence imaging (512×512 pixels) was performed with a SP5 LSM (Leica Microsystems). Objective: ×60. Scale bar: 5 µm.

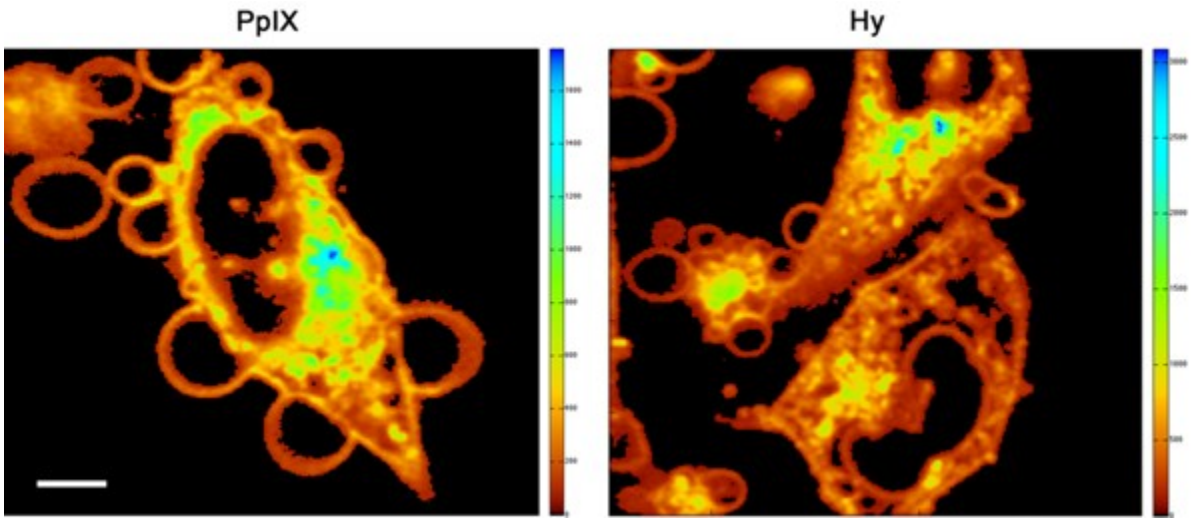

Figure S11. Photo-stimulation of PpIX or Hy induces a massive blebbing of the plasma membrane in Hela cells. After a 2 h incubation with PpIX-loaded LNC25 or Hy-loaded LNC25 (0.5 µM), HeLa cells were photo-irradiated with a Laser (405/488/590 nm wavelengths) for 5 min. FLIM images (256×256 pixels) indicate the localization of photosensitizers in the plasma membrane and the formation of big protrusions in this latter. Objective: ×60. Scale bar: 5 µm.