Supplementary Material

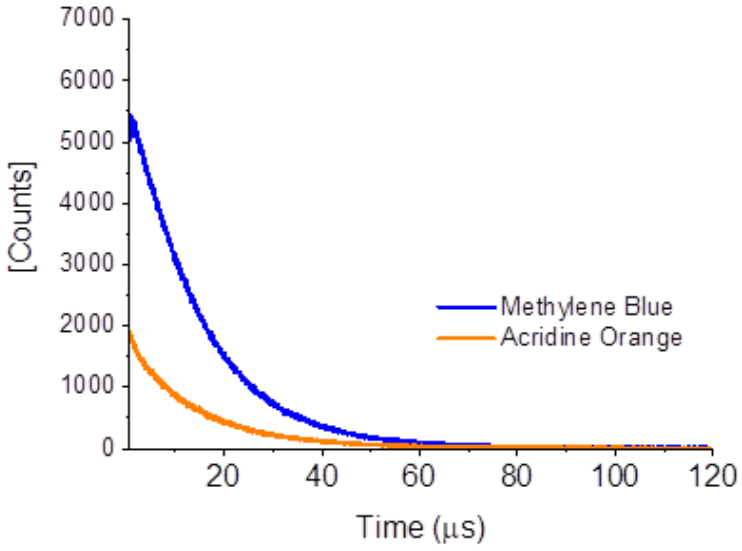

Figure S1. Transients of $^1O_2$ emission decays from acridine orange and methylene blue measured in ethanol. Excitation wavelength was 450 nm, and both solutions had equal absorbances at this wavelength. The signal was acquired in the near infrared, centered at 1270 nm, characteristic of singlet oxygen phosphorescence.

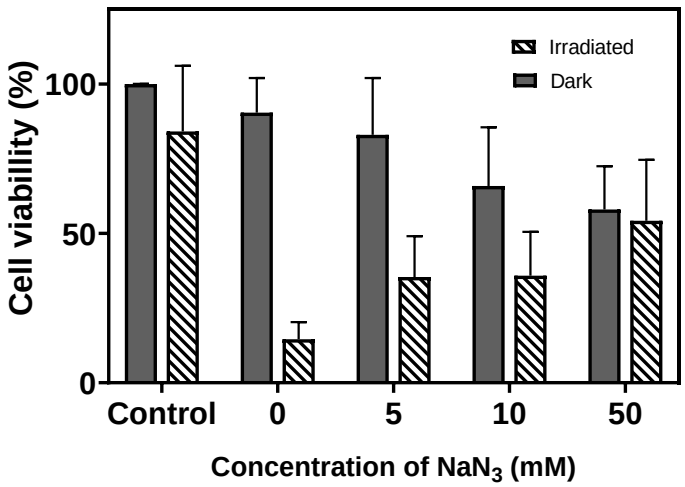

Figure S2. Viability test through the reduction of MTT by HaCaT cells for AO-PDT irradiated with blue light (λ=490 nm) in the absence and presence of sodium azide or in the dark (control). Cells were incubated with NaN_3 in the millimolar range (from 0 to 50mM) prior to irradiation.

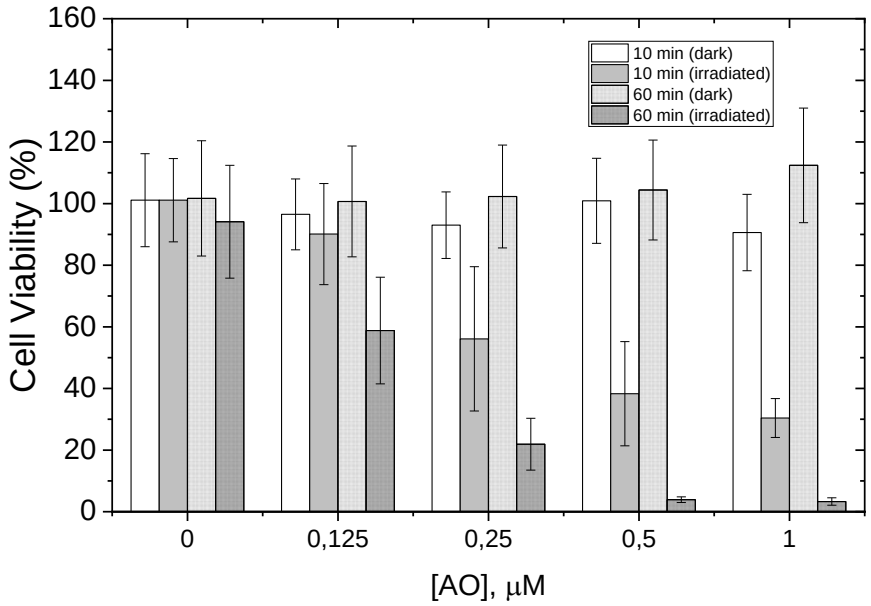

Figure S3. Cell viability assay by reduction of MTT by HaCaT cells, treated with AO for 10 min and 60 min in the range of 0 - 1 µM, incubated in the dark or irradiated with blue light (490 nm).

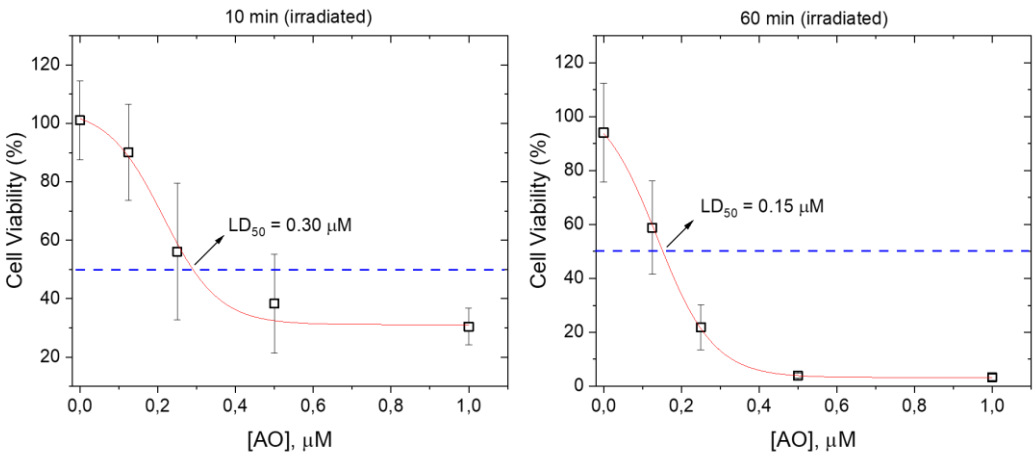

Figure S4. Lethal doses (LD_{50}) of AO incubated in HaCaT cells for 10 min (left) or 60 min (right) and then irradiated using the same protocol. There are differences in the LD_{50} values for the two incubation times, showing that this variable could influence the photoactivity of AO. The Dose Response curves were chosen using OriginGraph8 Software for fitting the data.

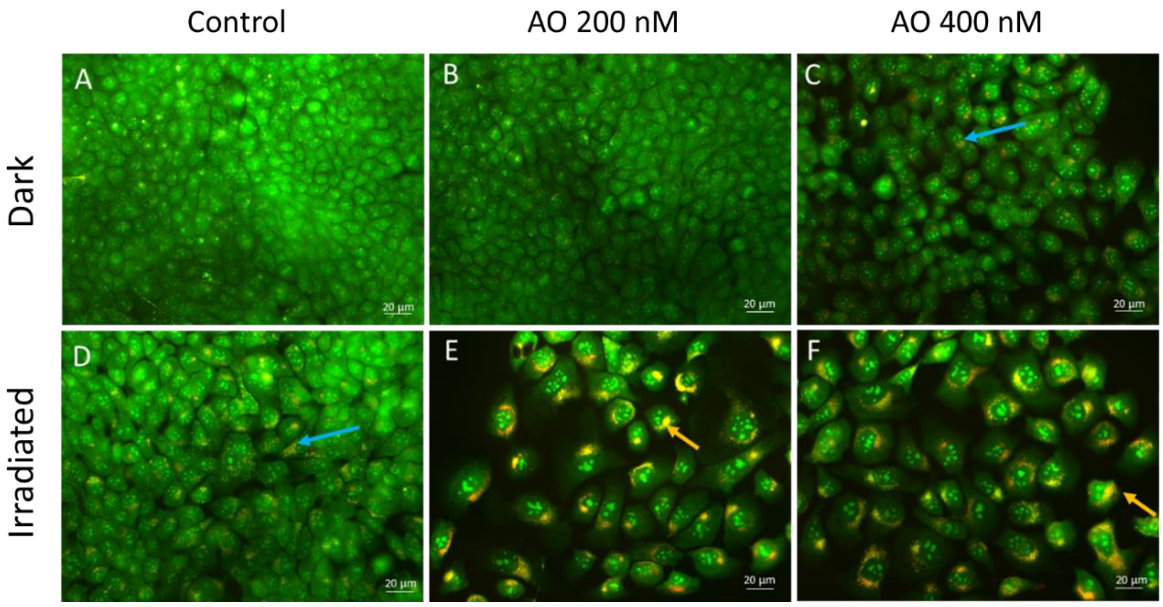

Figure S5. Fluorescence microscopy of HaCaT cells stained with 2.5 µM of AO 48h after PDT application with 10 min incubation with AO for identification of AVOs: (A) non-irradiated cells without PS, (B) non-irradiated cells with 200 nM PS, (C) non-irradiated cells with 400 nM PS, (D) cells irradiated without PS, (E) cells irradiated with 200 nM PS, (F) cells irradiated with 400 nM PS. 48

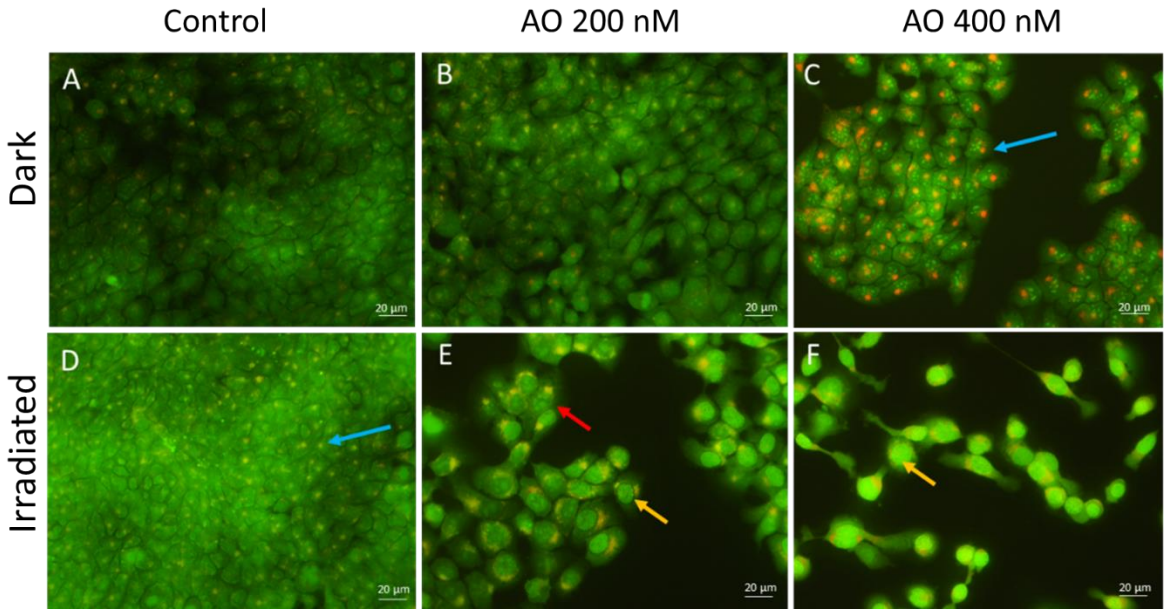

Figure S6. Fluorescence microscopy of HaCaT cells stained with 2.5 µM of AO 48 h after application of PDT with incubation for 60 min with AO for identification of AVOs: (A) non-irradiated cells without PS, (B) non-irradiated cells with 200 nM PS, (C) non-irradiated cells irradiated with 400 nM PS, (D) cells irradiated without PS, (E) cells irradiated with 200 nM PS, (F) cells irradiated with 400 nM PS.

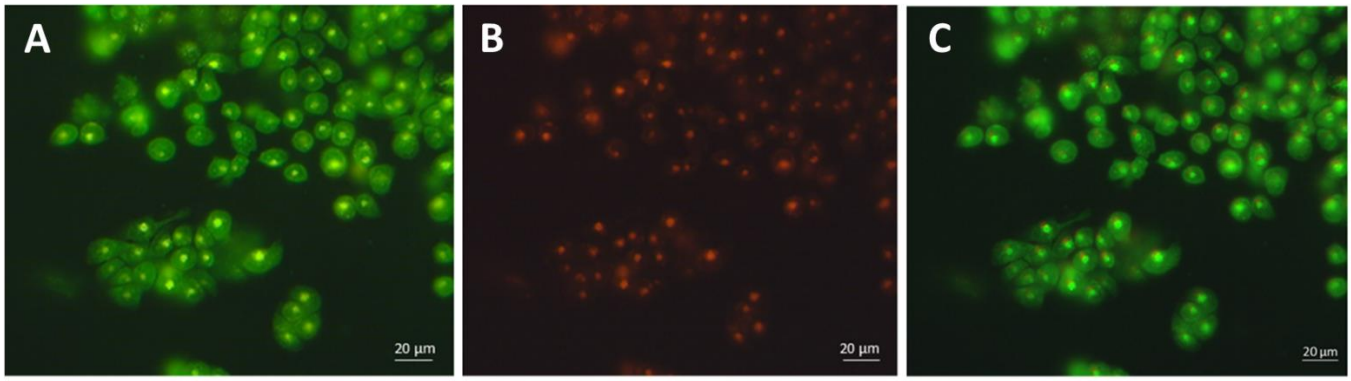

Figure S7. HaCaT cells incubated with 400 nM AO (A) or 100 nM of LDR (B), and the colocalization profile (C). Photographs were obtained with FS09, FS02 and FS20 filters for images A, B and C, respectively.

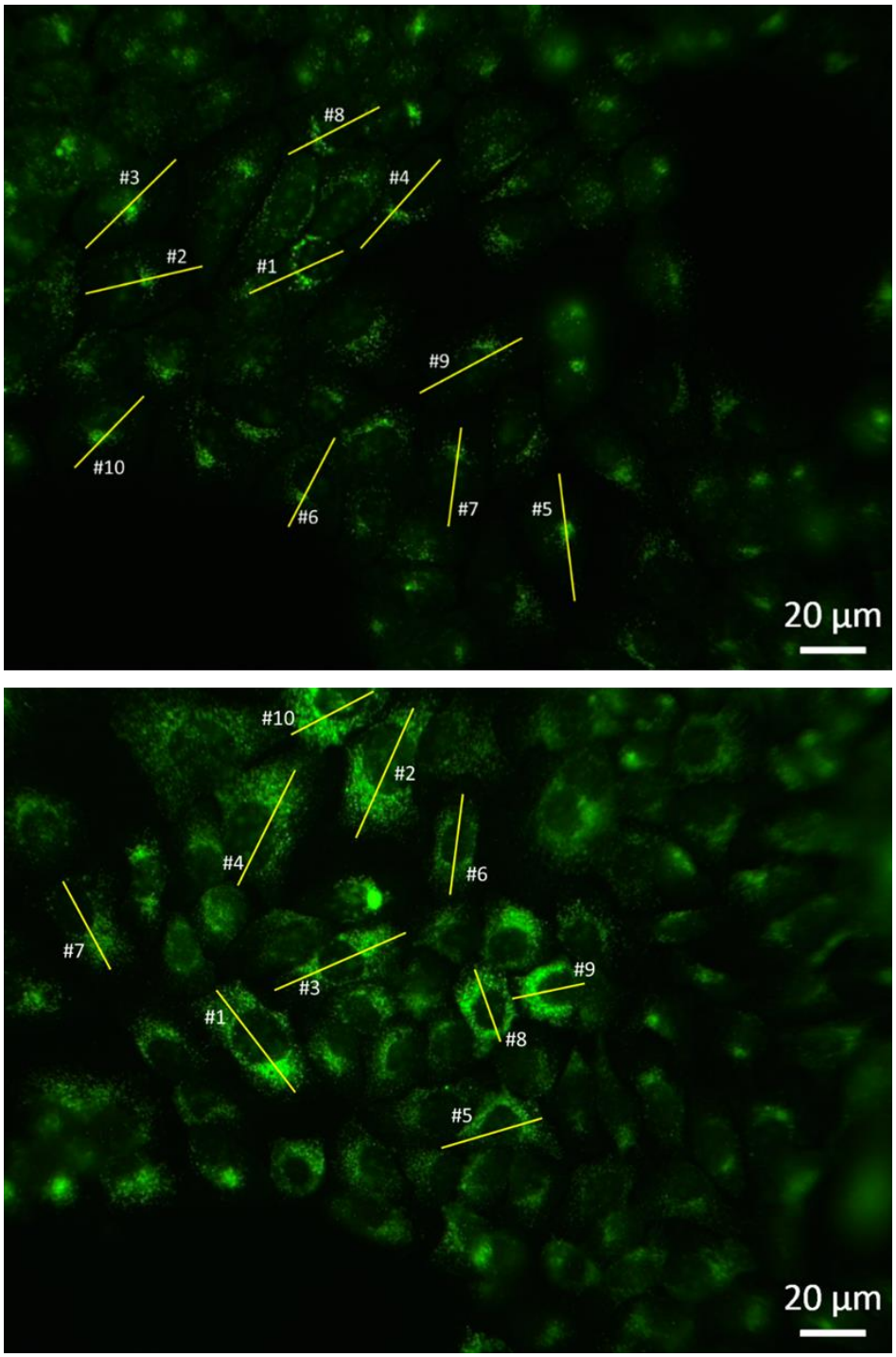

Figure S8. Epifluorescence images of HaCaT cells incubated with 400 nM AO for 10 min (top) or 60 min (bottom). The yellow traces indicate the 10 cells chosen to generate profiles in order to evaluate the intensities of fluorescence in each scenario and show the differences in the distributions of AO at the two times of incubation. Each cell provided a profile of fluorescence intensity like that in Fig. 4 (bottom), and hence, the averaged intensities were used to quantify the luminescence arising from the cells.

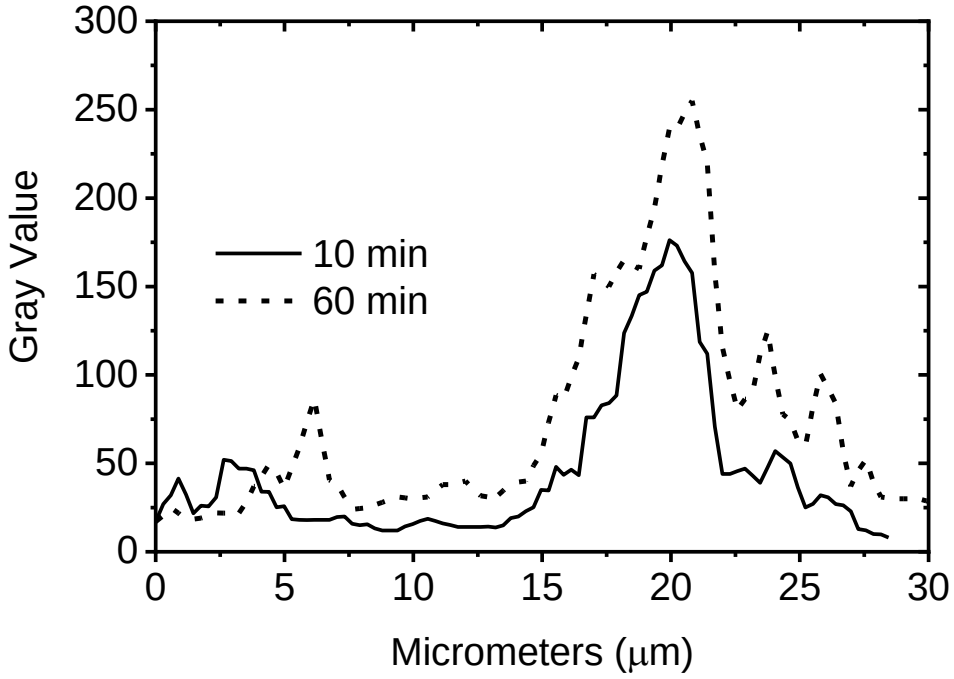

Figure S9. Profiles of fluorescence intensity from HaCaT cells treated with 400 nM AO for 10 min (solid line) or 60 min (dashed line), obtained from FLIM images. The longer incubation time shows more intense fluorescence.

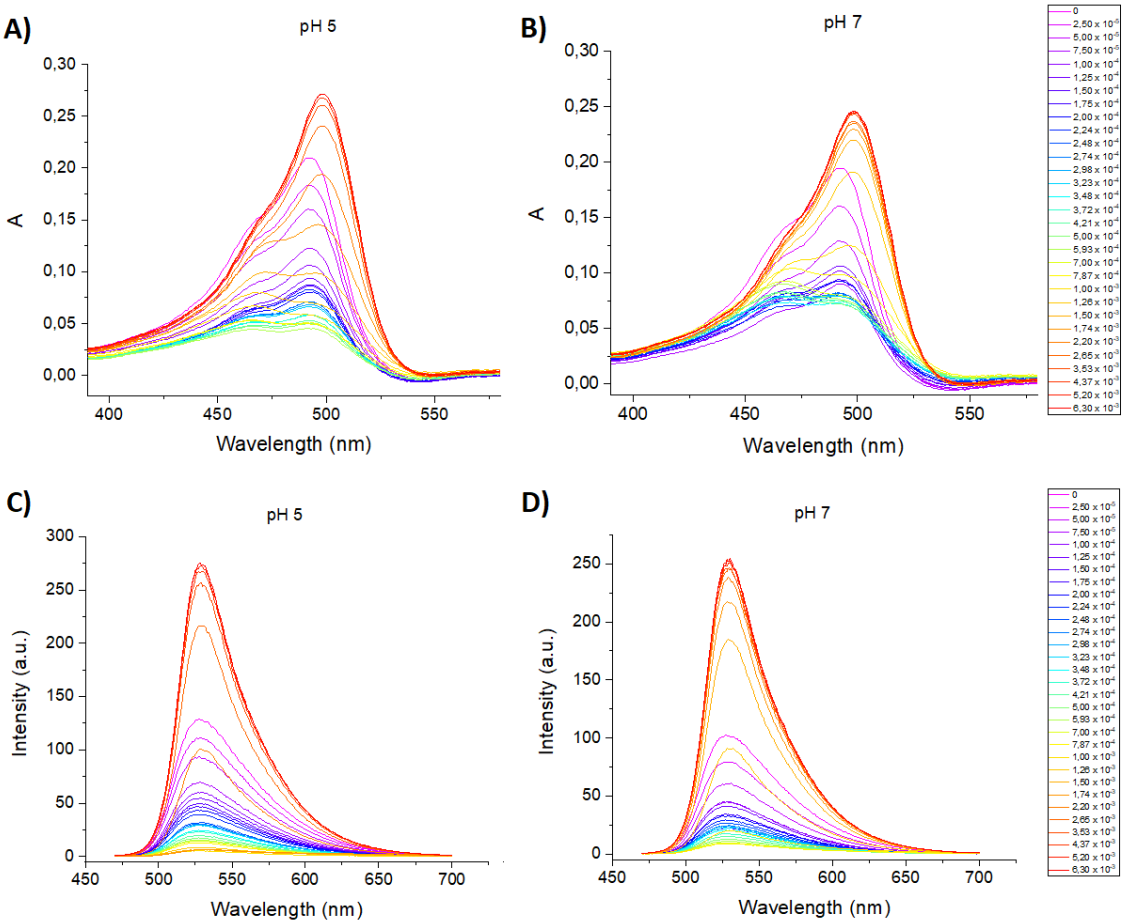

Figure S10. Absorption and fluorescence spectra of AO (5 mmol L^{-1}) titrated with 50 mmol L^{-1} SDS at pH 5 (A and C, respectively) or 7.4 (B and D, respectively).