Supplementary information

Supplemental text and materials and methods

Supplementary Text S1, Transfection studies:

PCI Transfection of AY27 cells Using Poly-L-Lysine and p-EGFP Preparation of DNA Complexes

Plasmid/poly-L-lysine complexes with charge ratio of 2.2 were formed by gentle mixing of plasmid DNA and poly-L-lysine solutions. 2.5 µl of DNA (stock solution 2 µg/µl) was diluted with 47.5 µl water, and 6.92 µl poly-L-lysine (1 µg/µl) was diluted with 43.08 µl water. After mixing, the solution was incubated at room temperature for 30 min, then diluted with culture medium to a final volume of 1 ml and added to the cells (1 ml per well).

Photochemical Transfection

AY27 cells were seeded out into twenty-four well plates (Nunc, Denmark) at a density of 7.5 x 10^4 cells per well. After 6 h of incubation the photosensitizer ($TPPS_{2a}$, 0,4 µg/ml) was added and the cells were incubated for 18 h at 37°C, washed three times with culture medium and incubated in 1 ml photosensitizer-free culture medium containing the plasmid / poly-L-lysine complex for 4 h. Treated cells were washed once with the medium, and after addition of 2 ml of culture medium the cells were exposed to blue light from the LumiSource light source (PCI Biotech AS), with a wavelength maximum at 435 nm and at an irradiance of 13 mW/cm^2. The cells were incubated at 37°C for 2 days before analysis for EGFP-expression by flow cytometry.

Flow Cytometry Analysis

In order to study EGFP expression the cells were trypsinized, centrifuged, resuspended in 400 ml of culture medium and filtered through a 50 mm mesh nylon filter. Then the cells were analyzed on a Coulter Epics Cytometer (Beckman Coulter, Inc., Brea, CA, USA). For each sample 10^4 events were collected. EGFP was measured through a 510–530 nm filter after excitation with an argon laser (15 mW, 488 nm). Dead cells were discriminated from single viable cells by gating on forward scattering *versus* side scattering. The data were analyzed with CELL Quest Software (Becton Dickinson).

Chemotherapeutics

The photosensitizer $TPPS_{2a}$ (LumiTrans®) was provided from PCI Biotech AS, Oslo, Norway.

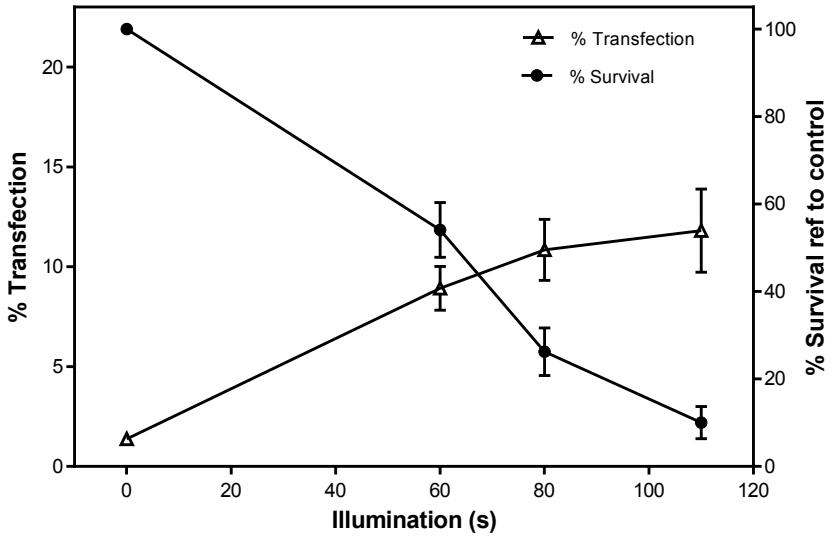
Supplemental Text 2. Results from Transfection Studies:

Transfection pEGFP in AY27 cells employing $TPPS_{2a}$

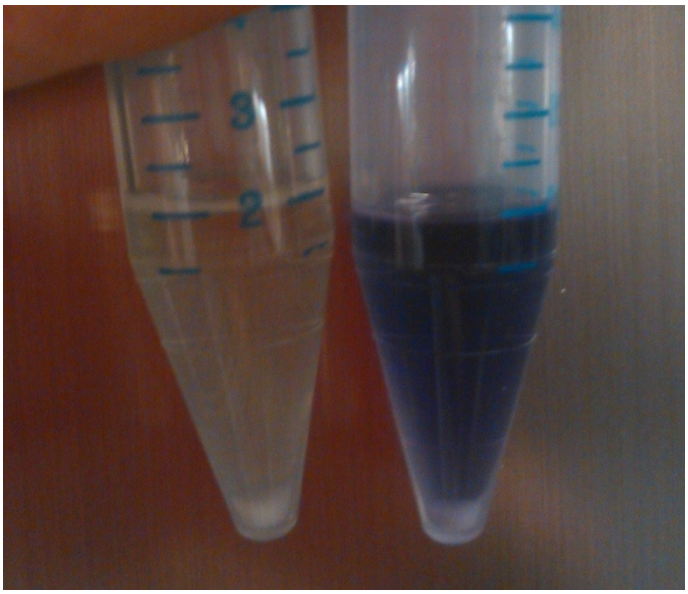
PCI-induced gene transfection experiments were performed with the pEFGP plasmid complexed to polylysine as previously described in the main paper employing the photosensitizer $TPPS_{2a}$, (LumiTrans®), and the presented in Supplementary Fig. S1 are transfection with pEGFP in AY27 cells employing $TPPS_{2a}$ (0.4 μg/ml) and illumination periods (s) as indicated in the figure. Cells were transfected as described under Supplementary Text S2 Material and Methods and the transfection was analyzed by flow cytometer 48h after illumination. Cell survival was measured by the MTT assay.

A significant enhancement of transfection was seen upon illuminations (Supplementary Fig. S1), increasing the percentage of EGFP-positive cells from 1% with no illumination to 12% at the

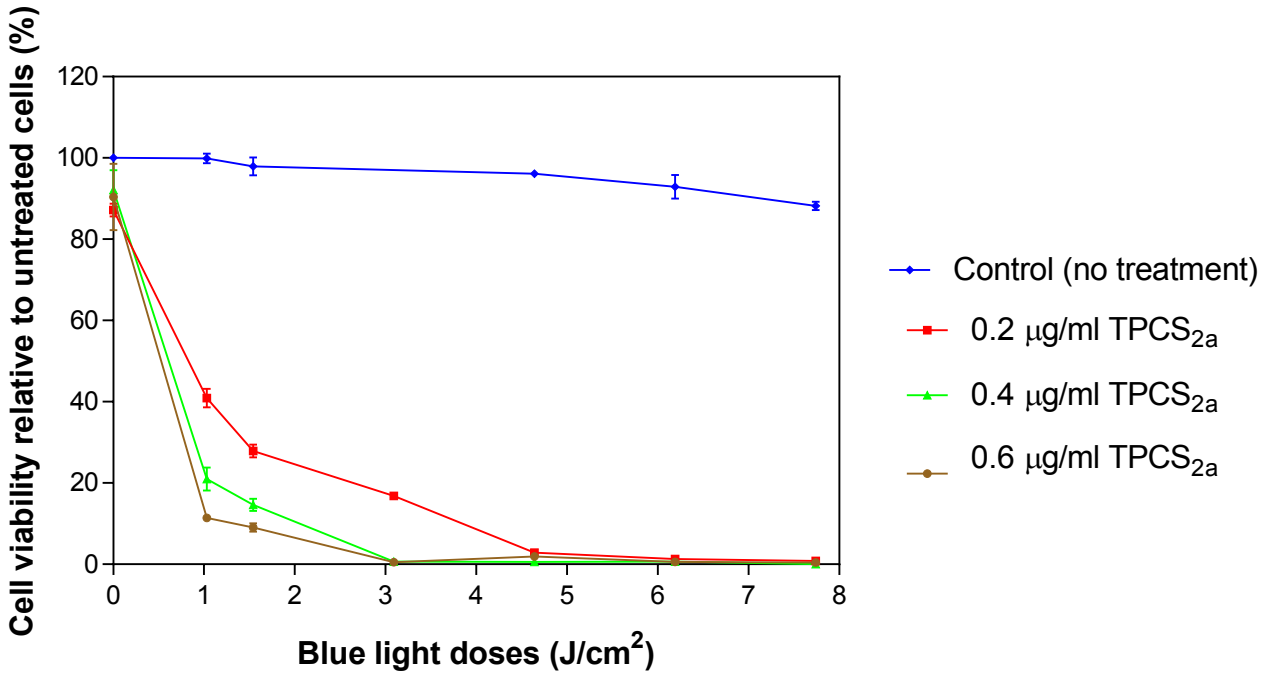
highest light dose and demonstrated that PCI induced gene transfection has effects on AY27 cells. As also reported for other cell types the enhanced transfection efficiency coincided with light induced cell toxicity with transfection efficiency levelling off at about 50% cell killing. In similar experiments, the two photosensitizers ($TPPS_{2a}$, $TPCS_{2a}$) have given comparable results in different cancer cell lines (unpublished results by Høgset et al., PCI Biotech AS, Oslo).



Supplemental Fig. S1 Transfection with pEGFP in AY27 cells employing $TPPS_{2a}$ (0.4 μg/ml) and light as indicated in the figure. Cells were transfected as described under Material and Methods, and the transfection was analyzed by flow cytometer 48h after illumination. Cell survival was measured by the MTT assay. Data are presented as mean value +/- SD of duplicates and represent one of totally three independent experiments and described in the main manuscript.



Supplemental Fig. S2 Presenting of cell debris, in 15 tubes, after an extra centrifuging step in MTT assay described by Charmical et al.[30] The clear supernatant after centrifuging (1500 rpm, 5 min) from untreated cells is present in the left tube and the blue formazan supernatant from PDT treated cells in the right tube. If necessary, further dilution was performed by isopropanol before spectroscopic measurement (Shimadzu UV-1700 spectrophotometer, 595 nm).



Supplemental Fig. S3 Cell cytotoxicity of AY27 cells after incubation with $TPCS_{2a}$ (0.2, 0.4 and 0.6 μg/ml, 18 h) before additional 4 h incubation in ordinary growth medium. Cell viability was examined by MTT assay post 24 h blue light exposure (LumiSource, 435 nm, as described in Section 2). The results are present as mean values +/- SD from three separate experiments.