

Supplemental information for:

Induction of immunogenic cell death in cancer cells by a photoactivated platinum(IV) prodrug

Vojtech Novohradsky,^a Jitka Pracharova,^{a,b} Jana Kasparikova,^a Cinzia Imberti,^c Hannah E. Bridgewater,^c Peter J. Sadler,^c and Viktor Brabec^{*,a}

^aCzech Academy of Sciences, Institute of Biophysics, Kralovopolska 135, CZ-61265 Brno, Czech Republic. ^bDepartment of Biophysics, Centre of the Region Hana for Biotechnological and Agricultural Research, Palacky University, Slechtitelu 27, CZ-78371 Olomouc, Czech Republic. ^cDepartment of Chemistry, University of Warwick, Coventry CV4 7AL, UK.

V.N. and J.P. contributed equally. *e-mail: brabec@ibp.cz

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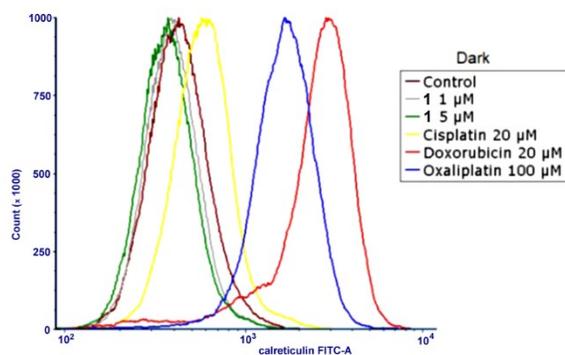


Figure S1. Analysis of ecto-CRT exposure after the treatment of A2780 cells with the indicated investigated compounds. A2780 cells were untreated or treated with the indicated concentration of the tested compound for 2 h in the dark; the samples were subsequently incubated for 22 h in the drug-free medium. The samples were analyzed by flow cytometry. Representative histograms from five independent experiments.

Table S1. Antiproliferative activity of the investigated compounds in A2780 ovarian cancer cells.^a

	IC _{50, 72h} ^b	Irradiated 420 nm	Dark
1		3.3 ± 0.8	≥150
Doxorubicin		1.6 ± 0.2	1.8 ± 0.4
Oxaliplatin		5.9 ± 0.1	5.7 ± 0.9
Cisplatin		6.7 ± 0.9	7.6 ± 0.5

^aThe cells were incubated for 2 h with the drugs in the dark or under irradiation conditions [90 min pre-incubation followed by irradiation with blue light (420 nm, 77 Wm⁻², 30 min)]; the samples were subsequently incubated for 70 h in the drug-free medium.

^bIC₅₀ values were determined by the MTT method.

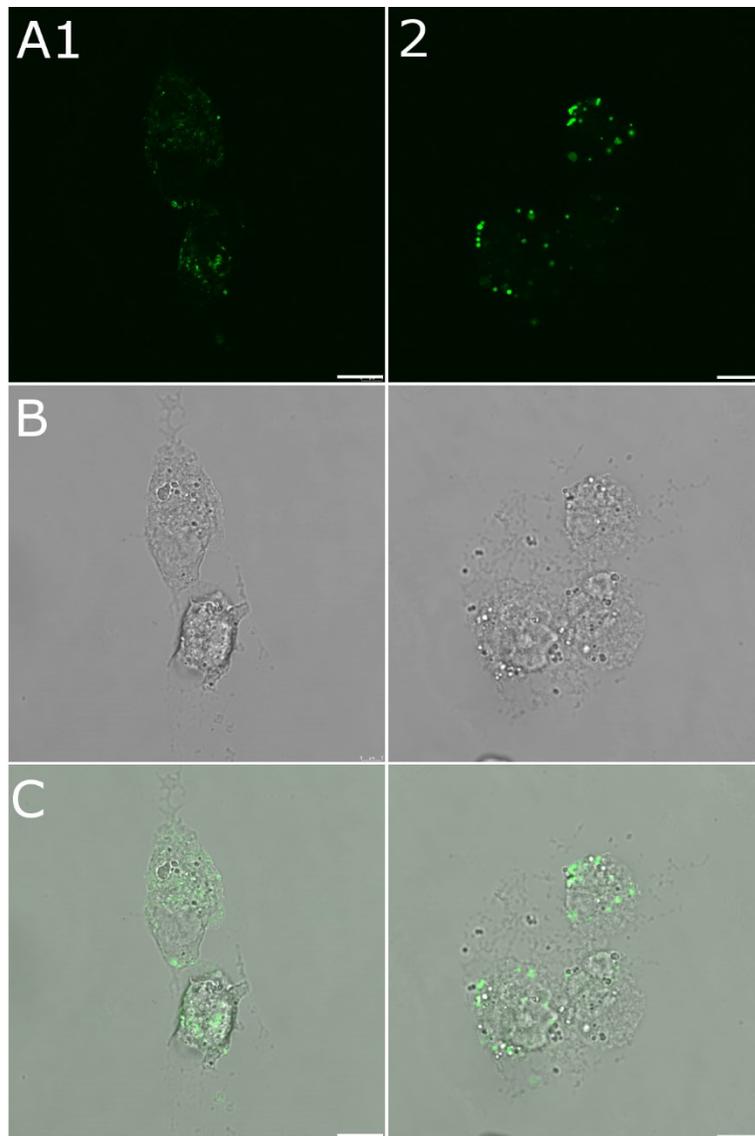


Figure S2. Immunofluorescence analysis of ecto-CRT exposure by confocal microscopy. A2780 cells were treated with doxorubicin (20 μM) (Panel 1) or with oxaliplatin (100 μM) (Panel 2) for 2 h in the dark followed by 22 h of incubation in the drug-free medium. Channels: A. Fluorescence signal from CRT-Alexa fluor 488 conjugate. B. Bright field. C. Merge of the fluorescence and bright field channels. Scale bars represent 10 μm.

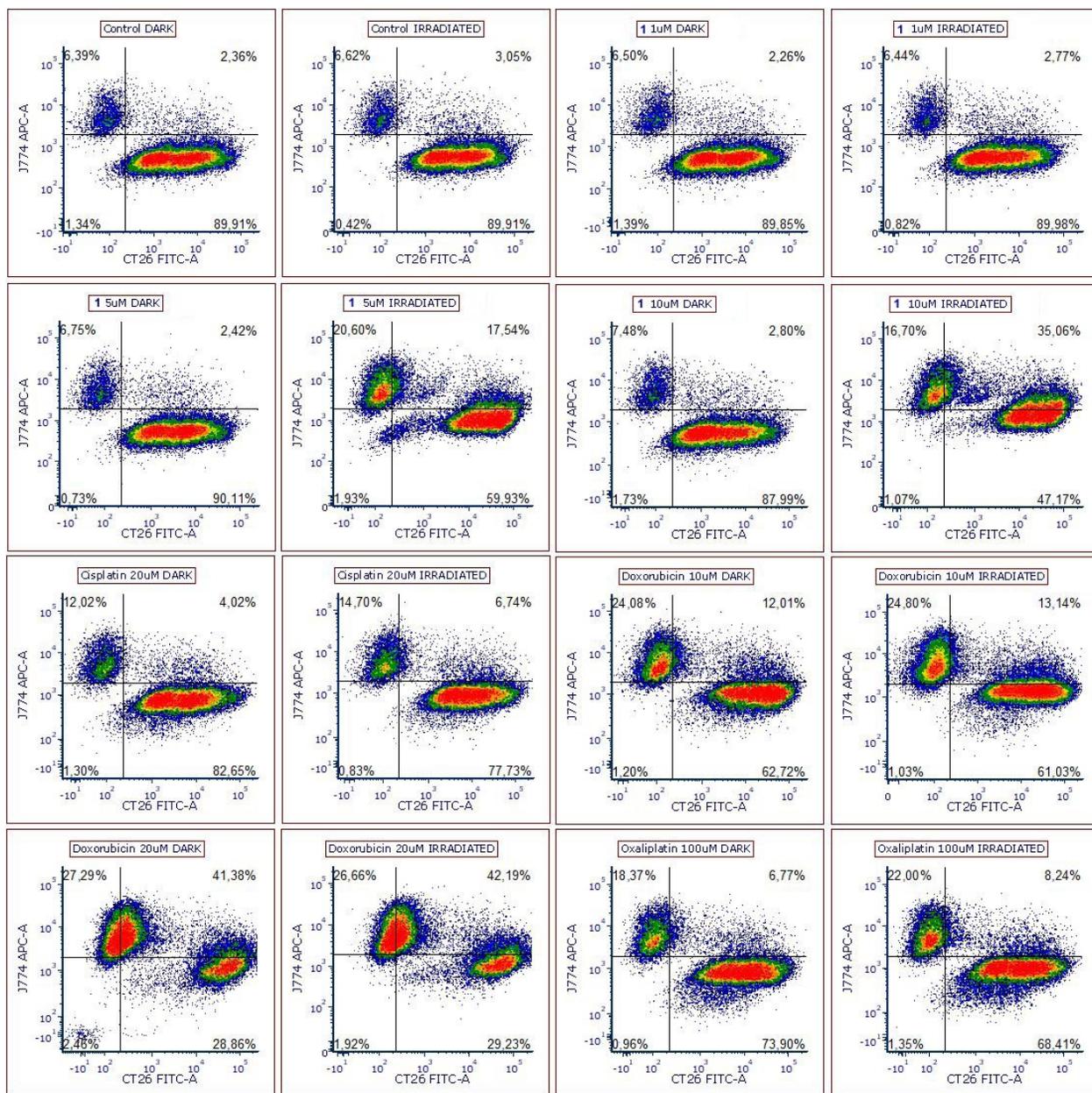


Figure S3. Analysis of phagocytosis induced in C26 carcinoma cells by **1**, doxorubicin, and oxaliplatin in the dark or under irradiation conditions. Flow cytometric analysis was performed using *in vitro* phagocytosis assay. Green-stained CT26 carcinoma cells were treated with the investigated compounds as indicated in the individual panels for 24 h and subsequently co-incubated with red-stained J774.A1 macrophages for 3 h. The percentage of phagocytosis was calculated from the number of double-stained macrophages over the total number of macrophages. The panels show representative flow cytometry density plots from three independent measurements.

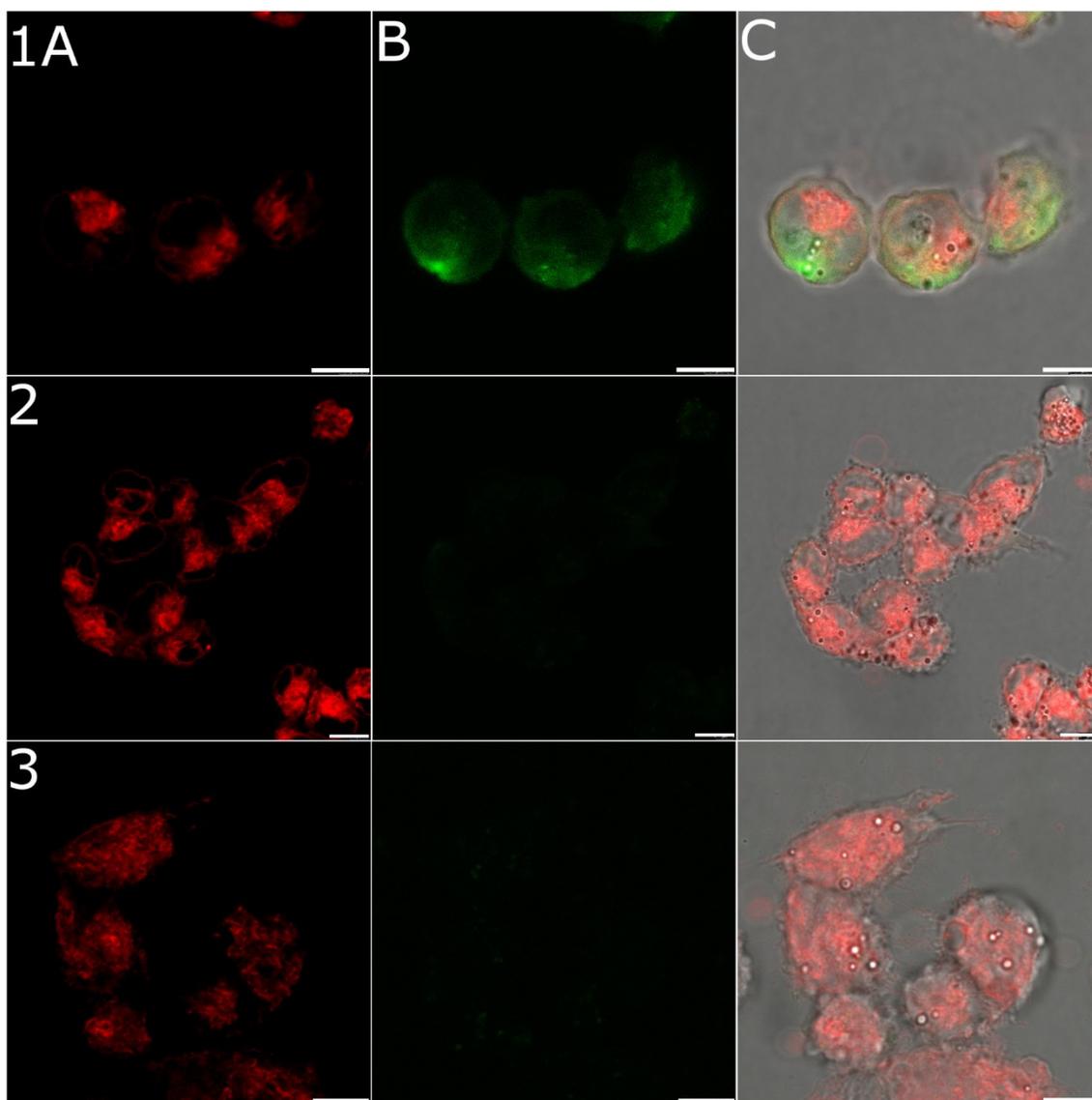


Figure S4. Detection of ROS in A2780 cells stained with ER tracker. Cells were treated with complex 1 at the concentration of 5 μ M (Samples 1, 2) or treated with vehicle control (Sample 3). Samples were irradiated with 420 nm blue light (Samples 1, 3) or kept in the dark (Sample 2). Panels: A) Red fluorescence channel for detection of the signal coming from ER tracker B) Fluorescence signal coming from CellROX detection probe C) overlay of the bright field, red and green fluorescence channel. Scale bar indicate 10 μ m.