Supporting Information

A dinuclear ruthenium(II) complex excited by near-infrared light through two-photon absorption induces phototoxicity deep within hypoxic regions of melanoma cancer spheroids

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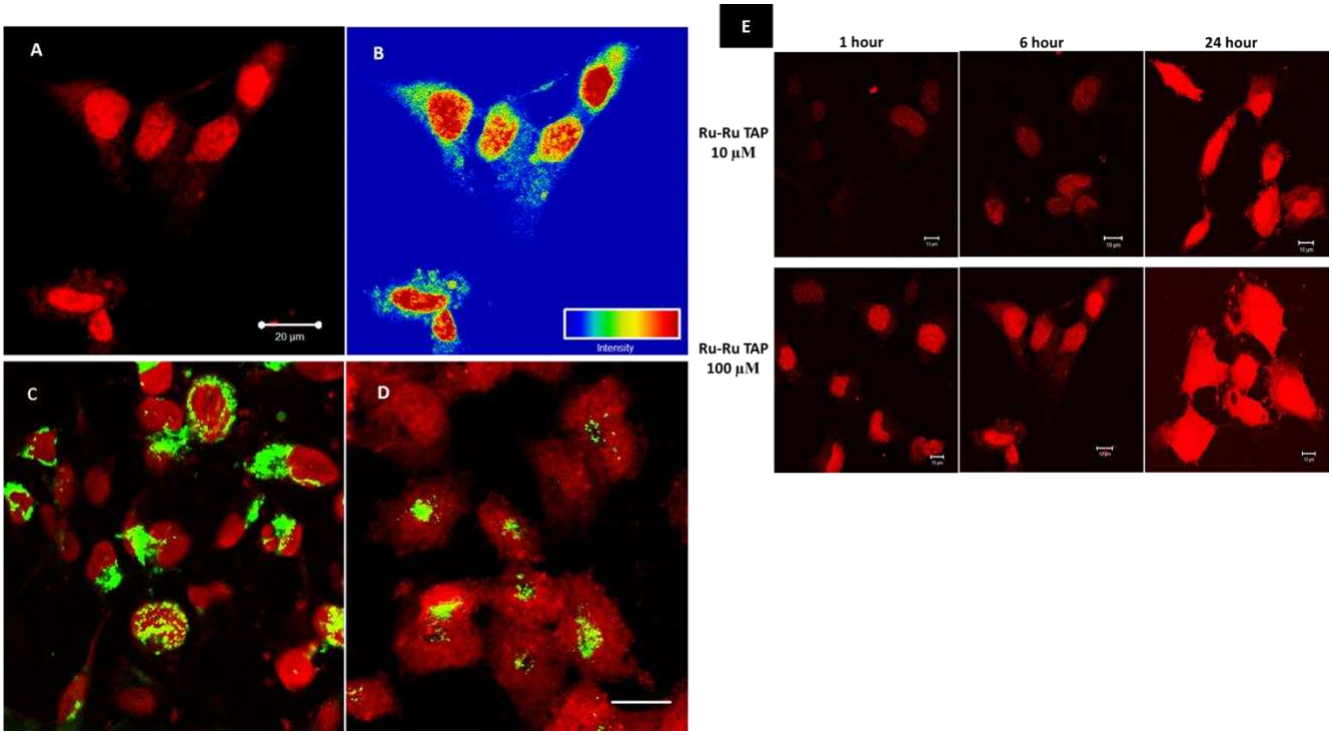
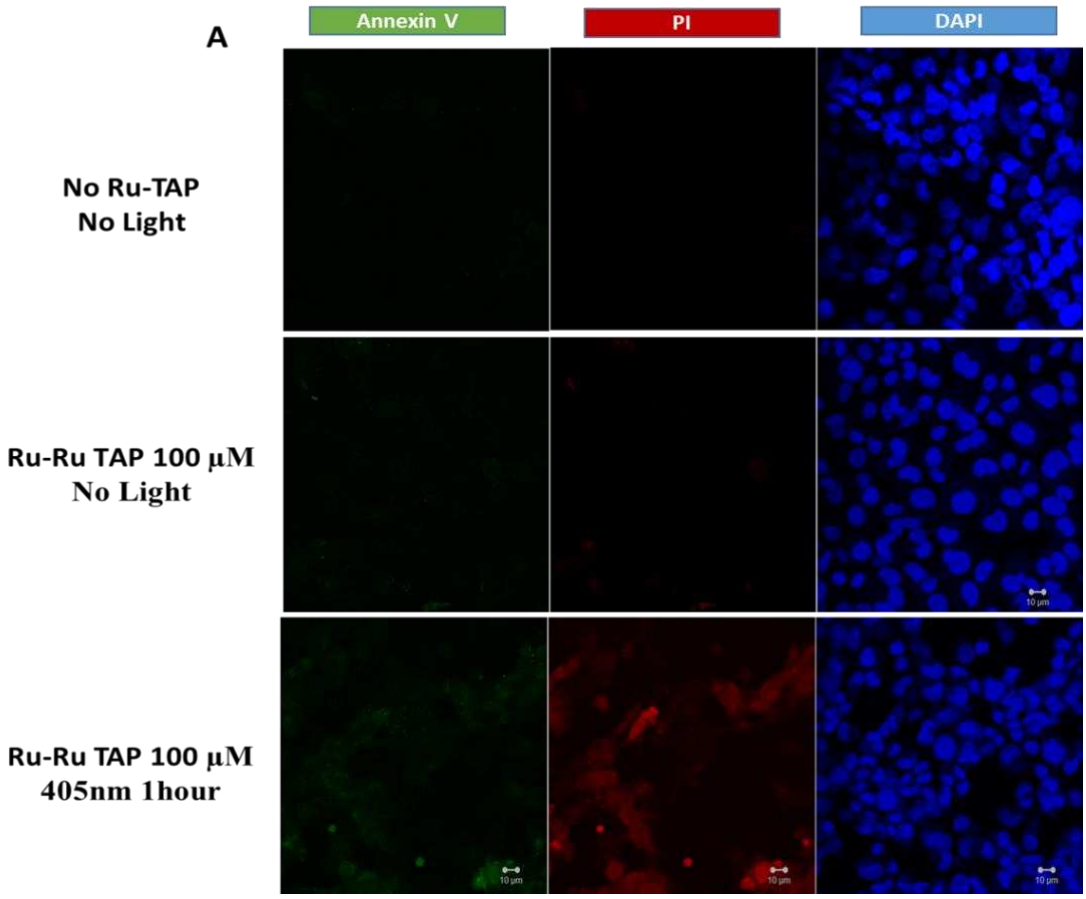


Figure S1: Intercellular localization and uptake of Ru-Ru TAP in human melanoma cells.
(A-B) Ru-Ru TAP compound predominate in the nucleus of human C8161 melanoma cells, that is also distributed within the cytosol (Pearson coefficient = 0.51±0.19, SB 20µm). **(C)** C8161 melanoma cells loaded with a mitochondria specific dye (white) and Ru-Ru TAP (red), observe punctate emission pattern of Mitotracker in the cytoplasm of cells that closely matches that of Ru-Ru-TAP emission (Pearson coefficient = 0.12±0.04) **(D)** Co-staining with Lysotracker (green) and Ru-Ru TAP (red). Observe the cytoplasmic emission of lysosomes and Ru-Ru TAP in the cytosol of melanoma cells, showing a partial dual emission suggesting lysosomal uptake (Pearson coefficient = 0.06±0.01, SB 20µm). **(E)** Progressive increase in uptake of Ru-Ru TAP by melanoma cells arises by increasing the concentration and exposure time. The varied exposure time does suggest that compound can readily diffuse into the cells and locates predominately in the nucleus of the cells, nevertheless it was eventually disseminated throughout the whole cell (scale bar = 10µm).


A
Annexin V
PI
DAPI
No Ru-TAP
No Light
Ru-Ru TAP 100 µM
No Light
Ru-Ru TAP 100 µM
405nm 1hour

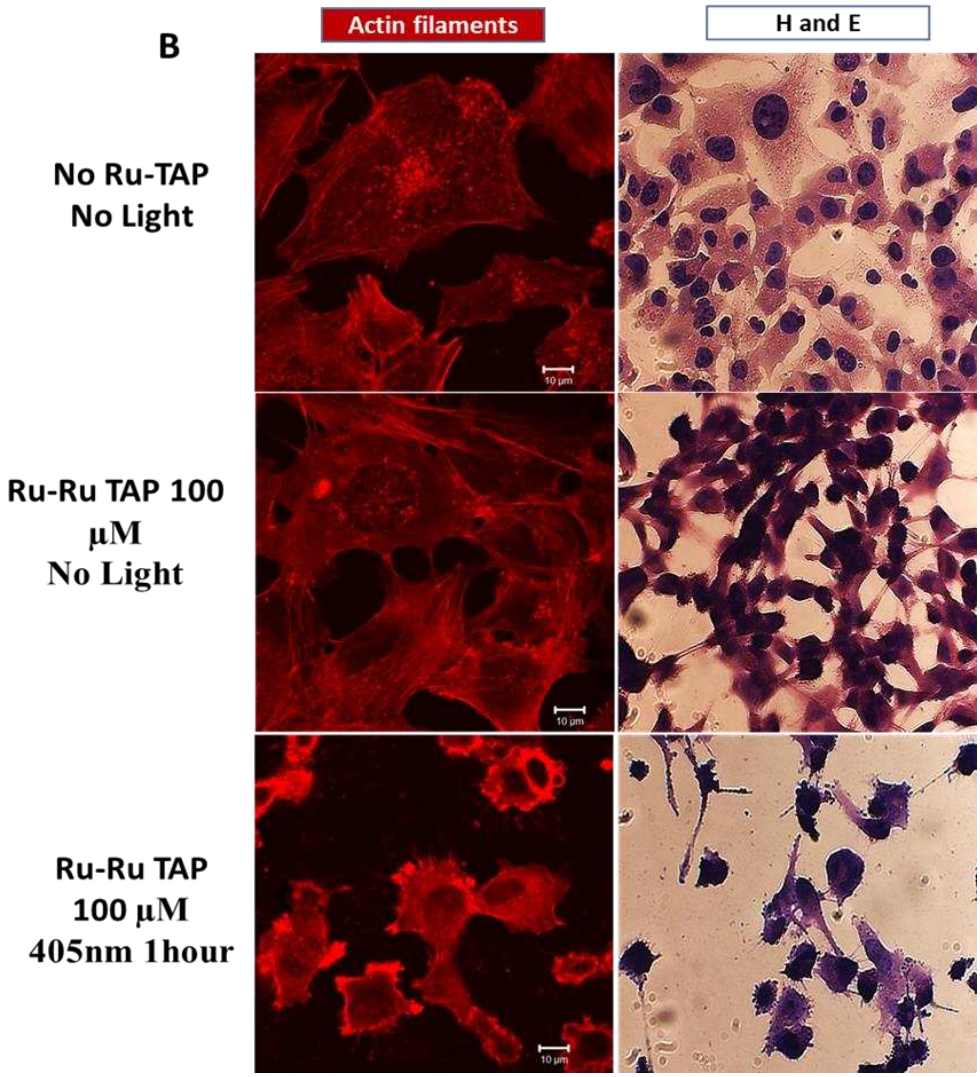


Figure S2: Apoptosis and/or necrosis caused by Ru-Ru TAP PDT on human melanoma cells.
Cell death can be in response to either apoptosis or necrosis pathways. Human melanoma cells (C8161) were treated with or without Ru-Ru TAP (100 µM for 24 hours) and activated with or without 1 hour (6.01J/cm2) 405 ± 20nm LED lamp. (A) The cells were then labelled for apoptosis (Annexin V-FITC, green) and necrosis (propidium iodide, red) detection. No apoptosis or necrotic cells were seen after only Ru-Ru TAP treatment or light treatment. When the Ru-Ru TAP treated cells were activated with light we noticed both apoptosis and necrosis within the cell population. (B) Phalloidin-TRITC (actin filament) and haematoxylin/eosin imaging of the melanoma cells after PDT (using the same conditions described above) showed shrinkage in cellular size, decrease in nuclear size and cell blebbing (scale bar = 10µm).

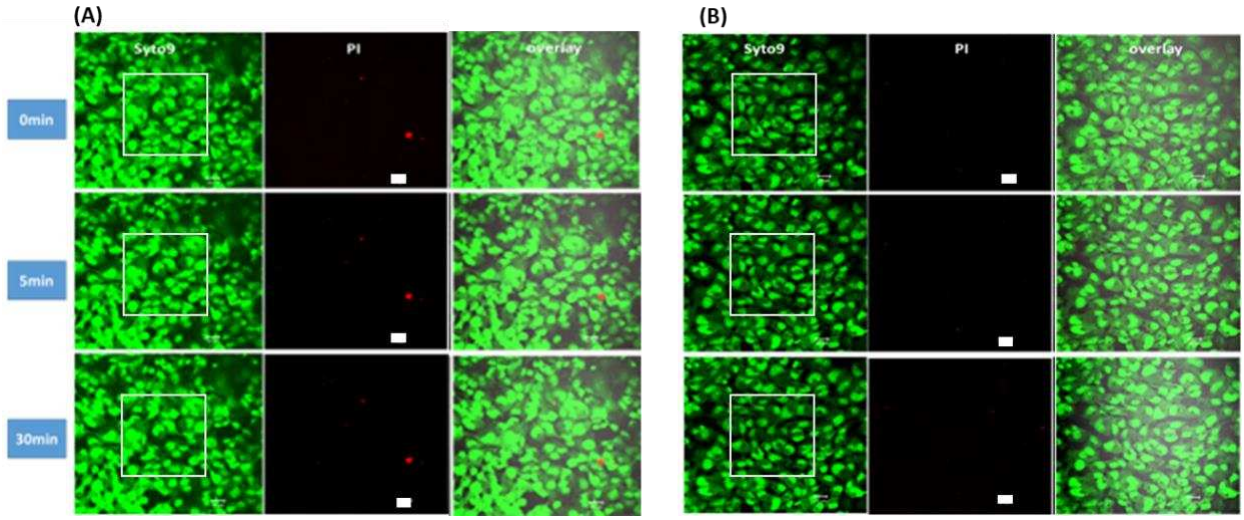


Figure S3: Two-Photon photo-toxicity without Ru-Ru TAP

Human melanoma cells were seeded on a 35mm dish plate ($5x10^5$ cells/well). After 24 hour incubation cells were washed with fresh serum free medium and replenished with live and dead medium (Propidium iodide (PI at 500 nM) and Syto-9 (2 µM) in SFM) for 15 minutes and through the length of time of the experiment. Live and dead cell images were taken from the same area (512 x 512 pixel) after every 5 minutes of irradiation at 900nm at (A) 10 mW and (B) 20 mW (scan speed = 6) on a marked region of interest (250 x 250 pixel). Irradiation was carried using a Ti:sapphire laser (Cameleon, Coherent) connected to a Zeiss LSM510 microscope using Achroplan (water dipping objective 40X, NA 0.75, WD 2.1) (scale bar = 20µm). No photo-toxicity was observed.

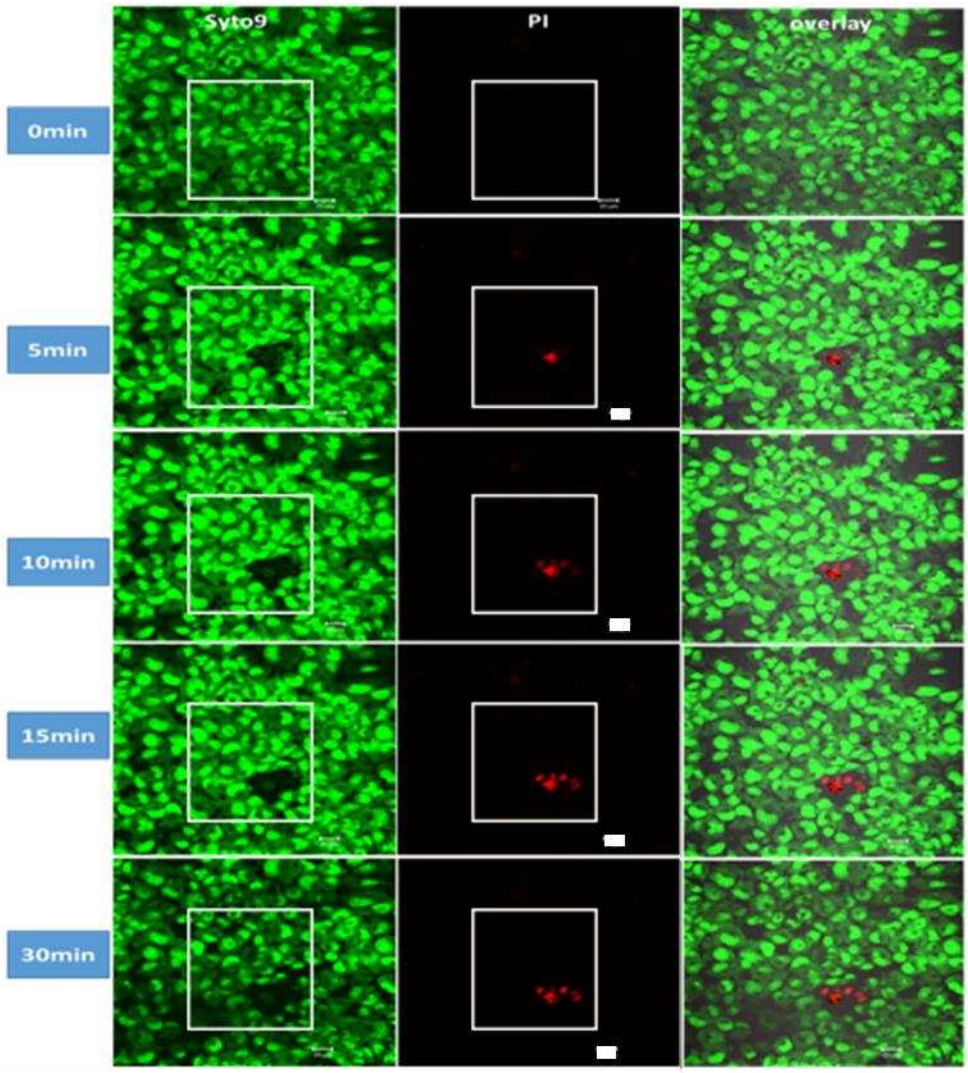


Figure S4: Two-Photon photo-toxicity with Ru-Ru TAP

Two-photon photo-toxicity of 1^{4+} in human melanoma cells treated with 1^{4+} (100 µM) after irradiation within the marked white square at 900nm (10mW, 30min). Live/dead cells imaged with Left column: Syto-9 (2 µM), Middle column: Propidium iodide (500 nM), and Right column: combined image. Recorded at 0, 5, 10, 15, 30 mins (scale bar = 20 µm).

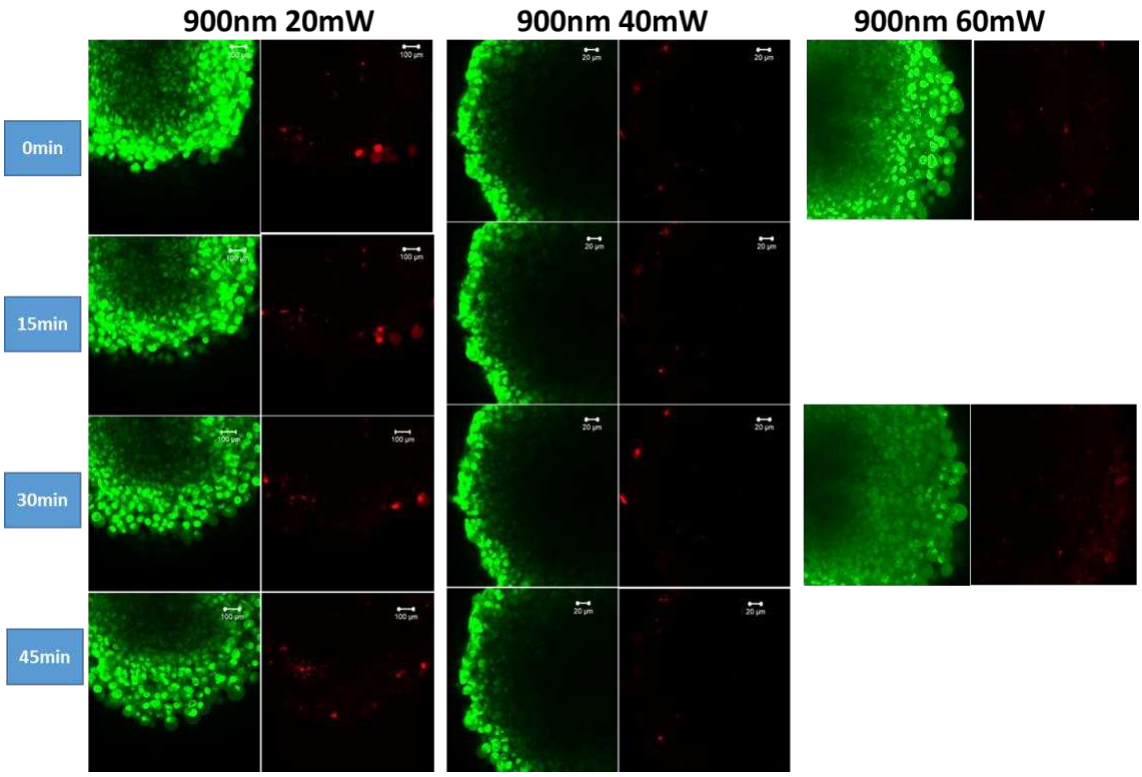


Figure S5: Two-Photon photo-toxicity in melanoma spheroids without Ru-Ru TAP
Human melanoma spheroids cultured for 10 days. Spheroids were allowed to settle on a 35mm plate overnight and incubated with propidium iodide (500nM) and Syto-9 (2µM) in serum free medium for 24 hours. Spheroids were irradiated with a 900nm laser (20, 40 and 60mW) using a continuous z-stack scan (10 µm apart) for 45 min. A live / dead cell scan was conducted through the whole spheroid (before and after each 15 min irradiance) (scale bar = 50µm).

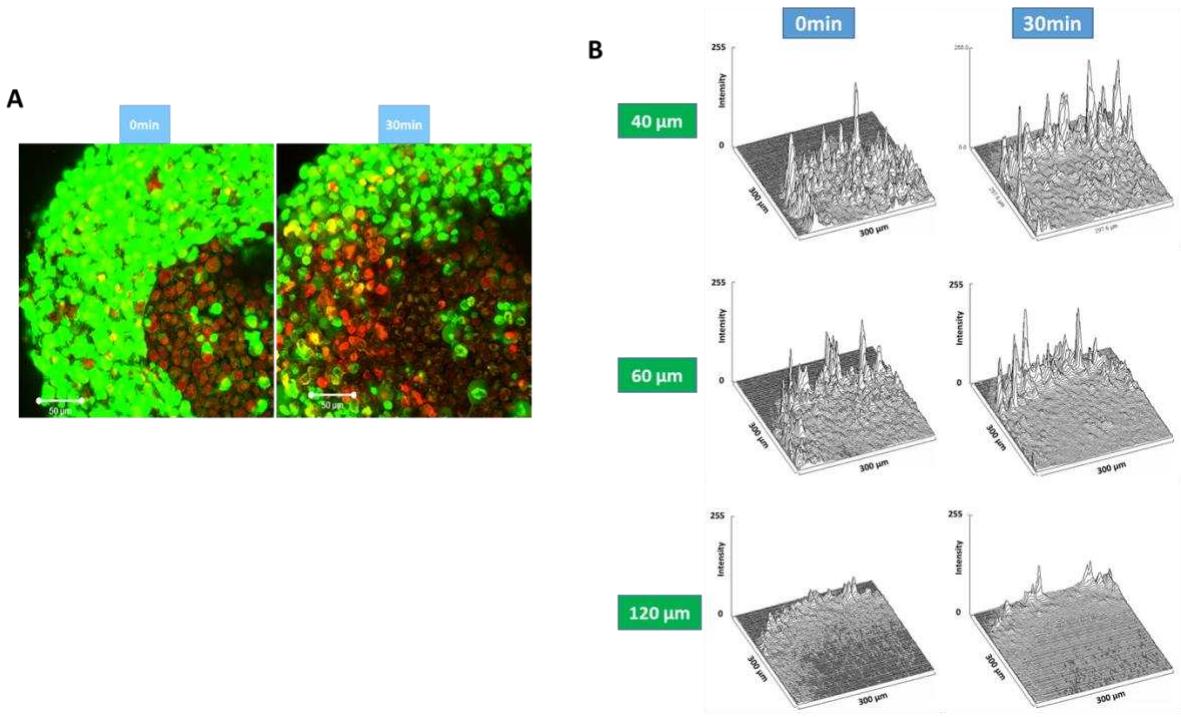


Figure S6: Two-photon PDT therapy of whole thickness of melanoma spheroids.

Human melanoma spheroids incubated with Ru-Ru TAP (100µM for 24 hours) irradiated with a 900nm laser (60mW) using a continuous z-stack scan (10µm apart) for 30 min, followed by live and dead cell scan through the whole spheroid (before and after every 15 min irradiance). (A) The entire z-stack data compiled into a single projection before (left) and after (right) illumination, revealing photo-induced cell death deep into the hypoxic layer (scale bar = 50 µm). (B) Note the increase in dead cell emission intensity with increase in irradiance time at each position (40, 60 and 120 µm).