**Supporting information**

**Mechanistic Investigation of Photodynamic Therapy using Visudyne in Human KB Carcinoma Cells**

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**2.1 Colocalization Experiment**

KB cells were co-incubated in RPMI media with 50 nM VD drug and 15 nM Mitotracker Green (obtained from Life Technologies) for 1 hour. Then cells were washed twice with PBS and kept in CO2 independent media (Life technologies, USA) for imaging. Since excitation of the VD drug may cause mitochondrial morphological changes within seconds, the Mitotracker green was captured at 488/520 nm and VD was captured at 690 nm.

**2.2 Singlet Oxygen Experiment**

Co-incubation of KB cells in FBS (-) MEM media with 50 nM VD drug and 10 µM Singlet Oxygen Sensor Green (SOSG, Life Technologies, USA) for 1 hour. After washing the cells twice with PBS and kept in CO2 independent media for imaging. Then irradiation of cells was done at 690 nm light followed by SOSG capture at 488/520 nm.

**2.3 ROS Experiment**

KB cells were incubated with 50 nM VD drug in RPMI media for 1 hour then washed twice using PBS. 30 min incubation of cells in RPMI media with 5 µM ROS followed by washing twice with PBS and kept in a CO2 independent media. Irradiation of cells at 690 nm light for photo-activating the VD drug followed by the ROS were capture at 488/520 nm.

**2.4 Cytoskeleton, mitochondria and DNA structure Experiment**

Incubation of KB cells with 50 nM VD drug in RPMI for 1 hour followed by washing twice with PBS and kept in CO2 independent media. For activating VD drug the cells were irradiated at 690 nm light. Fixation of cells using 4 % PFA for 15min, permeabilized with 1% Triton X-100 for 30 minutes and blocked with goat kit1 for 60 minutes. Stained the cells with F-actin green labeling kit (Life Technologies) for 30 minutes and 1µM Mitotracker deep-red FM (Life Technologies, USA) for 30 minute and 1 µg/mL Hoechst for 30 minutes. Cells were then washed twice with PBS and imaging captured using 520 nm emission wavelength for actin, 461 nm for Hoechst and 665 nm for mitochondria stain, respectively.

**2.5. Statistics**

Statistical analysis was performed using ORIGIN 8.6 program. To compare the population mean of different treatment groups at 0.05 significant levels the one way ANOVA was used. For statistical significance of mean difference among groups, Tukey Post Hoc test was further applied.

**Reference**

1. Y. Chen, N.P. Damayanti, J. Irudayaraj, K. Dunn, F.C. Zhou. Diversity of two forms of DNA methylation in the brain, *Front. Genet*. 2014, **10**, 46.