Title: Pyrroloquinoline Quinone (PQQ) Potentiates Chemo- and Radiosensitization of Cancer Cells in Synergistic way

Supplementary Data:

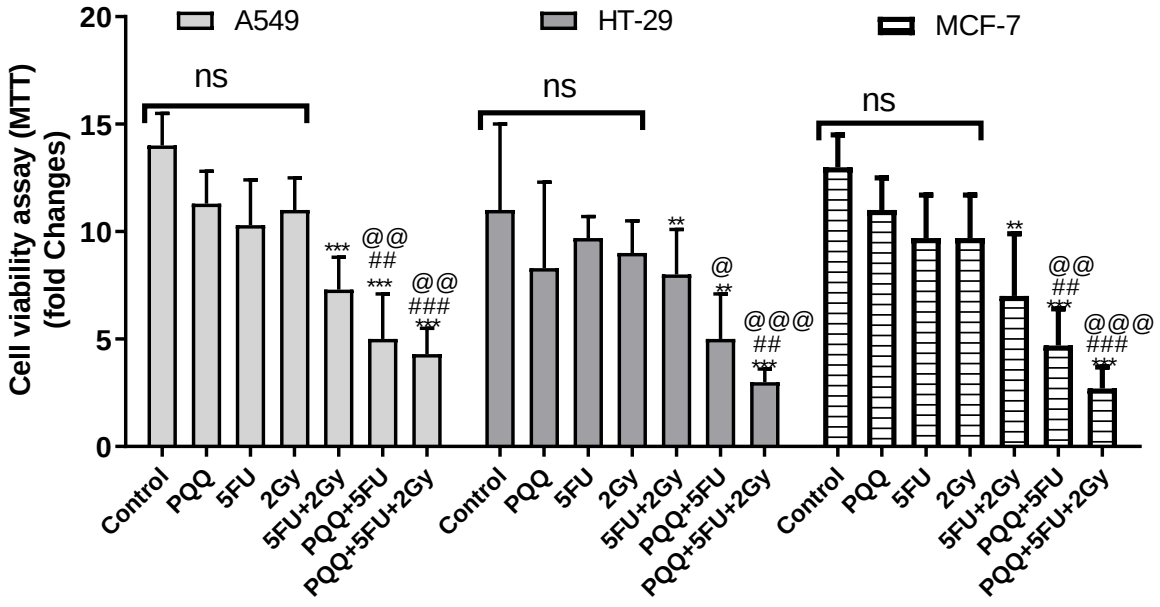


Figure S1. Effect of PQQ and 5-FU treatment of A549, HT-29 and MCF-7 cells on radiation-induced changes in cell viability by MTT assay (48 hours). Results are expressed as cell viability (fold changes) with respect to (Control vs different treatment*, PQQ alone vs different treatment $^{\#}$, drug alone vs different treatment $^{@}$ and PQQ+drug vs PQQ +drug +2Gy$^{\$}$), mean ± SD of three independent experiments. Statistical analysis of data was done using two-way ANOVA (analysis of variance) followed by post hoc analysis using Bonferroni post-tests (Prism 5.0), GraphPad (all columns were compared to different groups) unless otherwise stated. Differences were significant at values P< 0.05*, P<0.01**, P<0.001***, and were labelled with asterisks (*, #, @, $) and non-significant (ns).

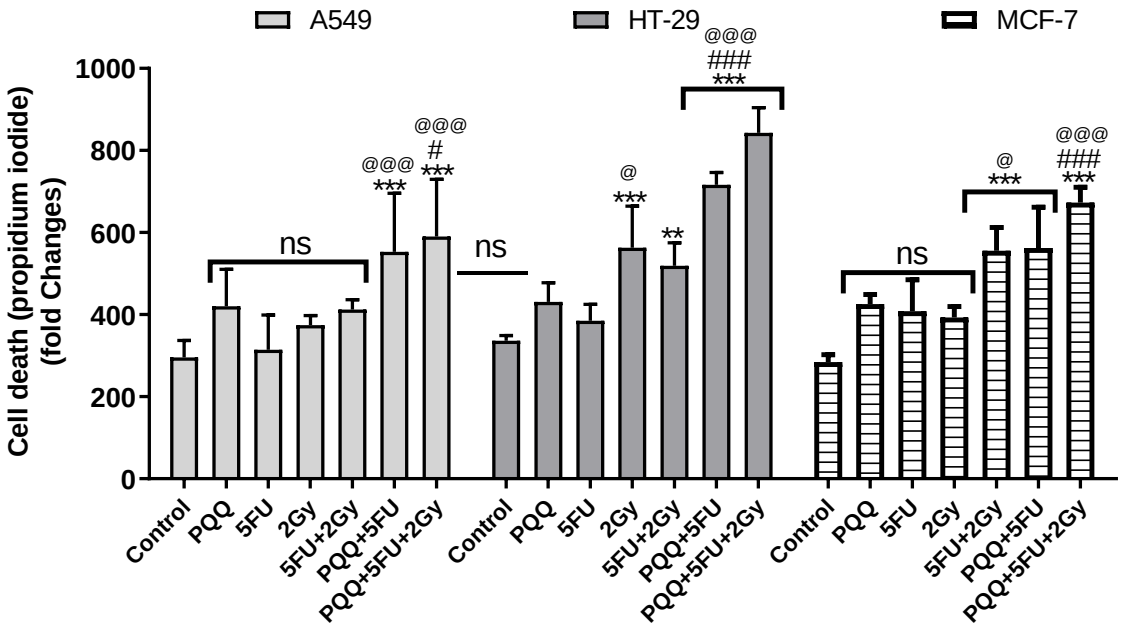
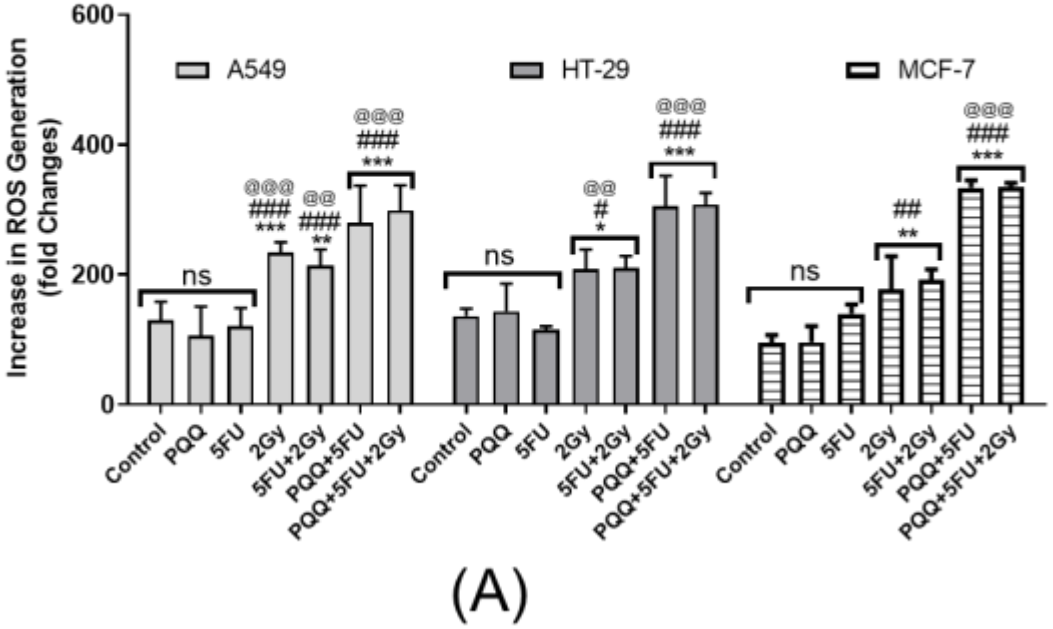
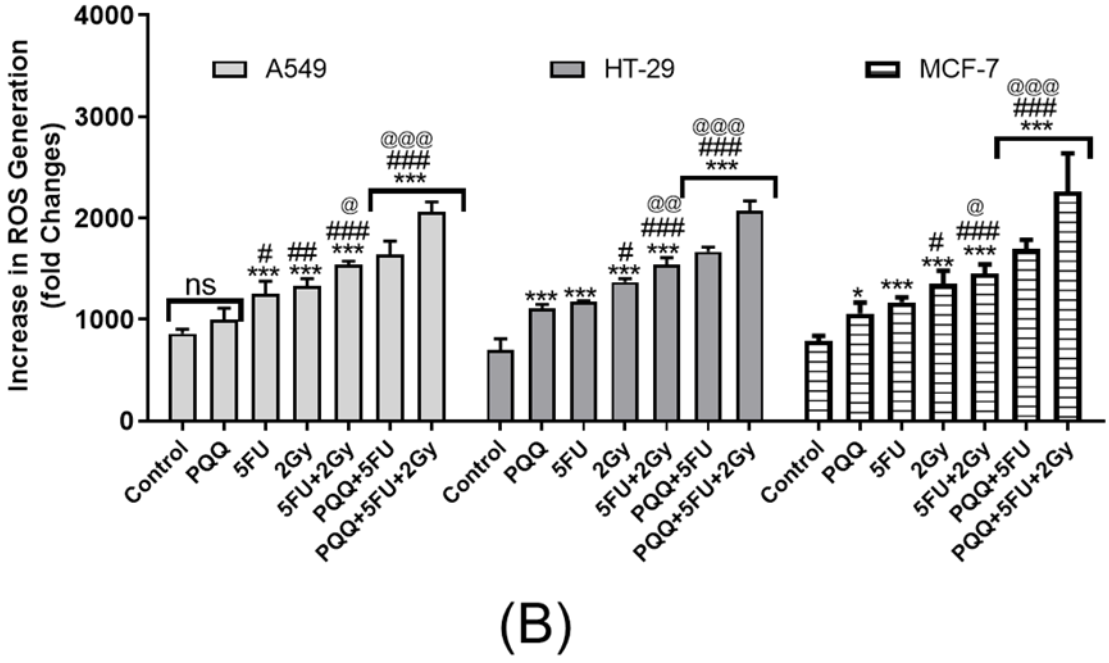


Figure S2. Propidium iodide (PI) staining measured with microplate reader. Relative fluorescence intensity at 615 nm is shown for different dead cells of A549, HT-29 and MCF-7. Values were measured using the microplate reader after 15 minutes staining with PI pre-stained cells. Error bars represent three individual repeats with three replicas. Results are expressed as Cell death by propidium iodide (fold changes) with respect to various treatment at (48hrs), Comparison was done among (Control vs different treatment*, PQQ alone vs different treatment$^{\#}$, drug alone vs different treatment $^{@}$ and PQQ+drug vs PQQ +drug +2Gy$^{\$}$), mean ± SD of three independent experiments. Differences were significant at values P< 0.05*, P<0.01**, P<0.001***, and were labelled with asterisks (*, #, @, $) and non-significant (ns). Statistical analysis of data was done using two-way (ANOVA) (analysis of variance) followed by post hoc analysis using Bonferroni post-tests (Prism 5.0), GraphPad (all columns were compared to different groups) unless otherwise stated.



(A)



(B)

Figure S3. ROS generation in A549, HT-29 and MCF-7 cells after 4hr (A) and 24hr (B). Dose dependent increase in ROS of various treatment with PQQ and 5-FU with gamma radiation. ROS mediated oxidation of CM-H_2DCFDA and measurement of the fluorescent CM-DCF with time (4-24hr) as an indicator of redox status of cells. Briefly, cells treated with PQQ, 5-FU, and 2Gy gamma radiation alone or PQQ in combination with 5-FU and 2Gy gamma radiation

were harvested and thereafter oxidation of dye was measured using λ_{Ex} 488 and λ_{Em} 530 as described under materials and methods. Obtained results presented are representative of three independent experiments. Comparison was done among (Control vs different treatment*, PQQ alone vs different treatment$^{\#}$, drug alone vs different treatment$^{@}$ and PQQ+drug vs PQQ +drug +2Gy$^{\$}$). Statistical analysis was performed by two way analysis of variance (ANOVA) with all pairwise multiple comparison procedures done by post hoc analysis using Bonferroni post-tests (Prism 5.0), GraphPad (all columns were compared to different groups) unless otherwise stated. Differences were significant at values P< 0.05*, P<0.01**, P<0.001***, and were labelled with asterisks (*, #, @, $) and non-significant (ns).Results are representative of mean fluorescence and expressed as fold changes.