

Gil del Alcazar et al., Legends to Supplementary Figures

Supplementary Figure S1. NVP-BEZ235 inhibits the DDR in U87-vIII cells. **A.** Cells were treated for 1 hour with NVP-BEZ235 (indicated doses) or DMSO before irradiation (10Gy). Cells were harvested 30 minutes after irradiation and nuclear extracts were assessed for phosphorylation of proteins by Western blotting with the indicated antibodies. **B.** Survival of U87-vIII cells after treatment with NVP-BEZ235 (100nM) or DMSO and radiation was quantified by colony formation assays. The fraction of surviving colonies (y-axis) was plotted against the corresponding radiation dose (x-axis). Error bars, S.E.M.

Supplementary Figure S2. NVP-BEZ235 impairs growth of U87-vIII tumors when given in conjunction with IR. **A.** Subcutaneous tumor-bearing mice at the end of treatment with IR alone or IR with NVP-BEZ235. Bar, 10mm. **B.** Tumors were excised 24 hr after dose 7 and TUNEL staining was performed on paraffin sections to assess levels of apoptosis. As a positive control, a mock-irradiated tumor section was treated with DNase.

Supplementary Figure S3. NVP-BEZ235 does not interfere with the anti-tumor effects of TMZ in U87-vIII tumors. Data from Figure 3A is re-represented to show individual tumor growth profiles for the TMZ+BEZ arm. Please note that 6 tumors (Non-responders; dashed red lines) behave like the TMZ-treated tumors (purple line) while 6 tumors (Responders; solid red lines) exhibit longer tumor regression.

Supplementary Figure S4. NVP-BEZ235 inhibits the DDR in GBM9 neurospheres. **A.** Bright-field microscopy image of GBM9 neurospheres. Bar, 60 μ m. **B.** Neurospheres were treated for 1 hour with NVP-BEZ235 (100 nM) or DMSO before irradiation (10 Gy). Cells were harvested 30 minutes after irradiation and nuclear extracts were analyzed for phosphorylation of proteins by Western blotting with the indicated antibodies. Survival of GBM9 neurospheres after treatment with NVP-BEZ235 (100 nM) or DMSO and radiation was quantified by colony formation (**C**) and sphere formation (**D**) assays. Neurospheres were irradiated at the indicated doses, dissociated into single cells 6 hours after irradiation, and plated onto 6 cm dishes in serum-containing medium for colony formation or suspended as single cells in stem cell medium in 96-well plates for sphere formation. The fraction of colonies or spheres formed (y-axis) was plotted against the corresponding radiation dose (x-axis). Error bars, S.E.M.

Supplementary Figure S5. Radiosensitization of normal human astrocytes (NHA) with NVP-BEZ235. NHAs were irradiated at the indicated doses and plated out for colony formation. The fraction of colonies formed (y-axis) was plotted against corresponding radiation dose (x-axis). Error bars, S.E.M. Please note lesser extent of radiosensitization, especially in the 2-6 Gy dose range, when compared to U87-vIII (Figure S1B) or GBM9 (Figure S4C) cells.