Supplementary Table 1. Enzymatic screen of PI-103 inhibition against a panel of 72 kinases.

Protein kinase assays were performed using a filter based radiometric platform in the presence of 3uM PI-103 and 10uM ATP.

Supplementary Figure 1. Continuous PI-103 treatment inhibits tumor cell clonogen outgrowth.

Single cell suspensions were plated, allowed to attach, and treated with PI-103 at the doses indicated. After 11-14 days growth in the presence of the inhibitor, cultures were stained and assessed for clonogen outgrowth. Results of PI-103 treatment are plotted relative to control cultures (clonogenic frequency of 1). Data are the average of duplicate points and are representative of replicate experiments.

Supplementary Table 2. Tumor cell clonogenic potential after 24 h PI-103 treatment.
Single cell suspensions were plated in replicate dishes for clonogenic survival. Once cells attached, 0.4μM PI-103 was added and drug exposure was maintained for 24 h after which culture medium was replaced with drug-free medium and cultures maintained for 10-14 day. Clonogenic survival changes after drug exposure were determined in replicate experiments and the mean decrease +/- sd is shown.

Supplementary Figure 2. Evaluation of DNA damage (γH2AX foci) after PI-103 treatment and irradiation.
Panel A: The effects of PI-103 on DNA damage foci at early times after irradiation were assessed by counting the γH2AX foci in cells fixed 30 minutes after 4 Gy irradiation. Cells were pretreated with PI-103 (0.4 μM x 1h) or LY294002 (10μM x

1h). Images were acquired on a GE InCell Analyzer (Cardiff, Wales) and values obtained after analysis on InCell Analyzer Developer software. Panel B: Western analysis of total cellular γH2AX from samples harvested 30 min 6h or 24h after treatment and irradiation as in panel A.

Supplementary Figure 3. Microscopic images of γH2AX and Rad 51 foci at the indicated times post-irradiation.

Foci were photographed in T24 cells (top) or SQ20B cells (bottom) irradiated after 1h treatment with PI-103 (0.4 μM), LY294002 (10μM) or DMSO (control). Cells were fixed at 30 min after irradiation with 2 Gy (30 min) or 24 h after irradiation with 4 Gy (24 h). The use of the lower dose at 30 minutes allows for better visualization of individual foci.