Supplementary Figure Legends

Supplementary Figure 1. Summary of testing and optimization of short-term cell survival assays. **A)** Illustration of 24-well plate format using syto60 staining. For each flat-well polystyrene plate (BD Falcon), untreated controls and seven positions for drug treatment are prepared allowing for triplicate technical repeats. Irradiated (2 Gy) and non-irradiated drug plates are always prepared, processed, and scanned in parallel. In pre-experiments, for each cell line an appropriate seeding density is determined so that at the end of the 3-day assay untreated control wells are no more than 80% confluent. Single-cell suspensions are prepared with the aid of a 19-gauge syringe as needed. In order to prevent cells from clustering at the edges or in the center of the wells, cells are carefully pipetted in the center of the well about a half inch above the bottom. After pipetting, plates are gently rocked to distribute cells evenly followed by incubation on a bench counter at room temperature for 20 minutes (see panel 1F). After 18-24 hours, new media with drug at final working concentration is added (and using 0.1% DMSO for untreated control wells), followed by 2 Gy X-irradiation or mock-irradiation 1 hour later. After 72 hours, cells are washed and fixed for 15 minutes with 3.7% formaldehyde. Following two more washes, plates are either stored at -4C or stained for analysis. For each experiment, a fresh batch of syto60 red fluorescent nucleic acid stain (5 mM stock solution in DMSO, in 50 μl aliquotes) (Invitrogen Cat.# S11342) is prepared. Cells are stained with 1 ml syto60 solution diluted 1:8,000 in PBS for 15 minutes, followed by PBS washes x 2. Plates are read by an Odyssey Li Cor Infrared Fluorescence Detection scanner. Images are converted to a TIF file, and the average intensity density of each well's surface area is measured using ImageJ. **B)** Three biological repeats using untreated duplicate plates of A549 cells. Plotted are meadian, quartiles, and range of fluorescence intensities per well. Data indicate that between plates handled in parallel (i.e., irradiated and non-irradiated) there is little random variation in staining intensity, while between biological repeats there can be considerable

variation in fluorescence intensity. **C)** Fluorescence variation across mean values of triplicate technical repeats per plate. Nine untreated plates growing A549 cells were analyzed. For each plate, data points represent the average fluorescence intensity of triplicate wells normalized to the average fluorescence intensity per plate (shown as horizontal line at Y=1). Bars represent standard deviation. Data suggest that in the majority of repeat experiments random variation of triplicate averages does not exceed +/- ~5% of the plate average, but that in cases where outliers may distort the results of a biological repeat (as in plates 1, 2, 5) more than 3 biological repeats are needed. Supplementary Table 1A shows that on average 4.6 biological repeats (range, 3-9) were obtained. **D)** Figure plots seeding density for cell lines shown against syto60 fluorescence intensity in a 24-well format, demonstrating a robust linear relationship across a range of seeding densities used in this study. **E)** Reduced well-to-well variation was observed when incubating plates for 20 minutes on a bench counter in room air following plating of DLD-1 cells, thereby allowing cells to adhere and not migrate to the outer well walls before placing the plates into the cell culture incubator. **F)** Average CellTiterGlo (CTG) luminescence intensity per row or per well in a 96-well plate. Marginal wells (i.e., rows 1 and 8, columns 1 and 12 shown as white bars) demonstrate reduced signal when an equal number of A549 cells was seeded and lysed for analysis five days later. Data suggest that outer rows and columns should be excluded in experiments. **G)** Triplicate well variations across the middle 60 wells in 96-well plates (without outer rows and columns excluded) comparing CTG, MTT, and Syto60 assays. Mean values were normalized to 1 for each plate. 95% confidence intervals are shown. **H)** Linear correlation between DLD-1 cell seeding number and relative signal intensity obtained with CTG, MTT, and Syto60 short-term assays (STA) in a 96-well format. STA intensity normalization was performed setting the signal from the lowest cell count to one. **I)** Quality control test for robotic CTG screening. Equal numbers of A549 cells were seeded into eight 96-well plates and grown for five days. The middle 60 wells were analyzed in triplicates (i.e., 20 triplicates in one plate). **J)** Definition of Short-term Radiosensitization Factor for 2 Gy irradiations (SRF_{2Gy}) using syto60-stained wells as an example. Indicated are the optical densities

obtained with ImageJ. FC, fraction of cells (corresponding to syto60-based fluorescence intensity). SRF_{2Gy} is the ratio of the FC for IR alone and the FC for combined drug/IR effect, corrected for FC observed for drug alone. SRF_{2Gy} values for CTG and MTT assays are calculated analogously.

Supplementary Figure 2. SRF_{2Gy} measurements and correlations. **A)** Correlation of SRF_{2Gy} based on syto60 assay with SRF_{2Gy} derived from clonogenic survival fractions following 2 Gy irradiation (CSA), analogous to Fig. 1E. **B)** Left, SRF_{2Gy} for cetuximab plotted for various drug incubation times prior to 2 Gy irradiation of Cal33 cells. Right, standard CSA for Cal33 treated with IR alone or with cetuximab added 1 hour prior to irradiation. Drug + IR curve was corrected for drug alone effect. Statistical comparison by F-test. Small SRF_{2Gy} and $DEF_{SF0.1}$ values of 1.03 and 1.05, respectively, correlate with in-vivo radiosensitization (see ref. 26). **C)** Plot of survival factions after 2 Gy (SF_{2Gy}) for 27 cell lines derived from the syto60 assay and the standard clonogenic survival assay (CSA), which shows lack of any correlation. **D)** SRF_{2Gy} values based on syto60 staining 3 days after 2 Gy irradiation and treatment with drugs against targets as indicated. NSCLC, non-small cell lung cancer; CRC, colorectal cancer; wt, wild-type; mut, mutant. **E)** Enhanced SRF_{2Gy} for various cell line-drug combinations when post-IR incubation times were extended to 6 days. Mean values with standard errors from at least three biological repeats were shown.

Supplementary Figure 3. Analysis of apoptosis after drug/IR treatments. **A)** Representative FACS histograms for NCI-H1703 and DLD-1 cells showing results of Annexin-V/propidium iodide double staining assay. Percentage of cells demonstrating early apoptosis (right lower quadrants) and late apoptosis/necrosis (right upper quadrants) are indicated. **B)** Percentage of Annexin-V positive NCI-H1703 cells for drug/IR combinations as indicated, with positive cells observed without any treatment or drug alone subtracted. **C)** Cumulative apoptosis as assessed by time-lapse light microscopy in NCI-H1703 cells +/- IR and +/- olaparib treatment. Representative images are shown on the right. 8,000

cells/well were plated in a 12-well plate. Following treatment, plates were placed on the moveable LUDL stage platform of a Nikon Eclipse TE 200S microscope (Melville, NY) located within an air-CO_2 incubator. Phase contrast images were taken every 20 minutes. Using Nikon Elements software, images were digitally streamed and apoptosis was manually quantified based on morphology. Approximately 60 cells along with their subsequent progeny were followed in a total of three repeat experiments. **D-E)** Quantification of apoptosis responses in DLD-1 and 639V cells with events in untreated or drug-alone treated cells subtracted. Bars represent mean values with standard error based on 2-3 biological repeat experiments.

Supplementary Figure 4. Analysis of senescence after drug/IR treatments. **A)** Representative images showing drug-induced SA-β-gal staining (highlighted by arrows) upon treatment of drugs against targets as indicated. Cells were stained after 3 days. DAPI images and SA-β-gal stained images were overlaid. Inserts indicate average percentage of SA-β-gal positive cells +/- 95% confidence limits based on 3 biological repeat experiments. **B)** Quantification of of SA-β-gal positive cells as a function of treatment as indicated in DLD-1 cells.

Supplementary Figure 5. Radiosensitizing effects of everolimus. **A)** SRF_{2Gy} values for isogenic *TP53* wild-type (wt) or mutant (mut, 273L) A549 cells treated with everolimus (20 nM). Statistical comparison by T-test. **B)** Clonogenic survival of isogenic p53 wild-type or mutant A549 cells following 6 Gy irradiation with or without everolimus. **C)** Left, representative anti-LC3 immunofluorescence images (40X) of A549 cells following treatment with 20 nM everolimus or 10 μM chloroquine (CQ) as a positive control for autophagy induction. Right, quantification of LC3 signal based on 10 random images with relative signal intensities normalized to untreated cells. **D)**

Quantification of LC3 signal in isogenic *TP53* wild-type or mutant A549 cells and *TP53* mutant PC9 cells following irradiation and with/without treatment with 20 nM everolimus.

Supplementary Figure 6. Radiosensitzing effects of midostaurin. **A)** Percentage of *KRAS*-mutant (mut) and wild-type (wt) NCI-H1703 cells with at least 20 subnuclear 53BP1 foci at 24 hours following 6 Gy irradiation +/- treatment with midostaurin and the DNA-PKcs inhibitor NU7026 (10 μM) as indicated. Numbers indicate fold-change resulting from addition of midostaurin. Data suggest that any effect of midostaurin on DNA double-strand break repair is independent of DNA-PKcs-mediated end-joining. **B)** Representative Annexin-V / propidium iodide flow histograms for isogenic NCI-H1703 cells with or without mutant (mut) K-ras expression analogously to Fig. S3A. **C)** Representative SA-β-gal staining images in isogenic DLD-1 cells either wild-type (wt) or mutant (mut) for KRAS. Arrows highlight SA-β-gal positive cells. **D)** Percentage of A549 cells with residual IR-induced 53BP1 foci following treatment with midostaurin and transfection with either control siRNA or siRNA against PKCα complementing Fig. 5E. **E)** Representative FACS histograms of CD133 sorting of A549 cells. Cells were incubated with monoclonal CD133/1 (CD133)-PE conjugate antibody (1:11) at 4°C in dark for 30 minutes. The cells were then analyzed and sorted by flow cytometry. Autofluorescence controls were run in parallel. A gate for "high" CD133 expressors was set to include 0% cells in the unstained population and ~2.5% in the stained population. **F)** SRF_{2Gy} values for NCI-H703 spheres which were drug treated and 2 Gy irradiated 3 days after seeding into 96-wells, followed by another 3 days of incubation in drug before lysis and analysis for CTG intensity. Bars represent mean +/- standard error for 3 biological repeat experiments.