SUPPLEMENTAL INFORMATION

Supplementary Table 1. Dose at which the survival fraction falls to 10% ($D_{10\%}$) calculated from the α and β values determined from the linear quadratic model. Uncertainties were determined from error propagation theory.

	$D_{10\%}$ (Gy)			
Cell Line	**Photon**	**1.1 keV/μm**	**2.5 keV/μm**	**7.3 keV/μm**
HT1080-shRAD51^{IND}	8.0 ± 0.3	7.06 ± 0.05	6.12 ± 0.08	4.69 ± 0.07
HT1080-shRAD51^{IND} + dox	4.04 ± 0.09	4.19 ± 0.07	3.84 ± 0.09	3.22 ± 0.03
HT1080-shDNA-PKcs	2.85 ± 0.03	2.60 ± 0.04	2.49 ± 0.04	2.39 ± 0.04
M059K	5.41 ± 0.16	6.03 ± 0.10	5.22 ± 0.03	4.02 ± 0.05
M059J	1.48 ± 0.04	1.653 ± 0.013	1.54 ± 0.10	1.38 ± 0.09
HCC1937	2.64 ± 0.26	—	—	1.318 ± 0.053
HCC1937-BRCA1	3.4 ± 0.55	—	—	2.06 ± 0.22

Supplementary Table 2. Dose at which the survival fraction falls to 50% ($D_{50\%}$) calculated from the α and β values determined from the linear quadratic model. Uncertainties were determined from error propagation theory.

	$D_{50\%}$ (Gy)			
Cell Line	**Photon**	**1.1 keV/μm**	**2.5 keV/μm**	**7.3 keV/μm**
HT1080-shRAD51^{IND}	3.78 ± 0.18	3.62 ± 0.03	2.71 ± 0.12	2.41 ± 0.12
HT1080-shRAD51^{IND} + dox	1.26 ± 0.10	1.44 ± 0.08	1.43 ± 0.11	1.14 ± 0.02
HT1080-shDNA-PKcs	1.14 ± 0.04	0.92 ± 0.04	0.86 ± 0.04	0.75 ± 0.02
M059K	1.95 ± 0.16	2.42 ± 0.14	1.89 ± 0.04	1.62 ± 0.06
M059J	0.445 ± 0.013	0.70 ± 0.02	0.46 ± 0.03	0.43 ± 0.04
HCC1937	0.76 ± 0.26	—	—	0.426 ± 0.05
HCC1937-BRCA1	1.02 ± 0.55	—	—	0.62 ± 0.22

Supplementary Table 3. Survival fraction at 2 Gy (SF_{2Gy}) calculated from the α and β values determined from the linear quadratic model. Uncertainties were determined from error propagation theory.

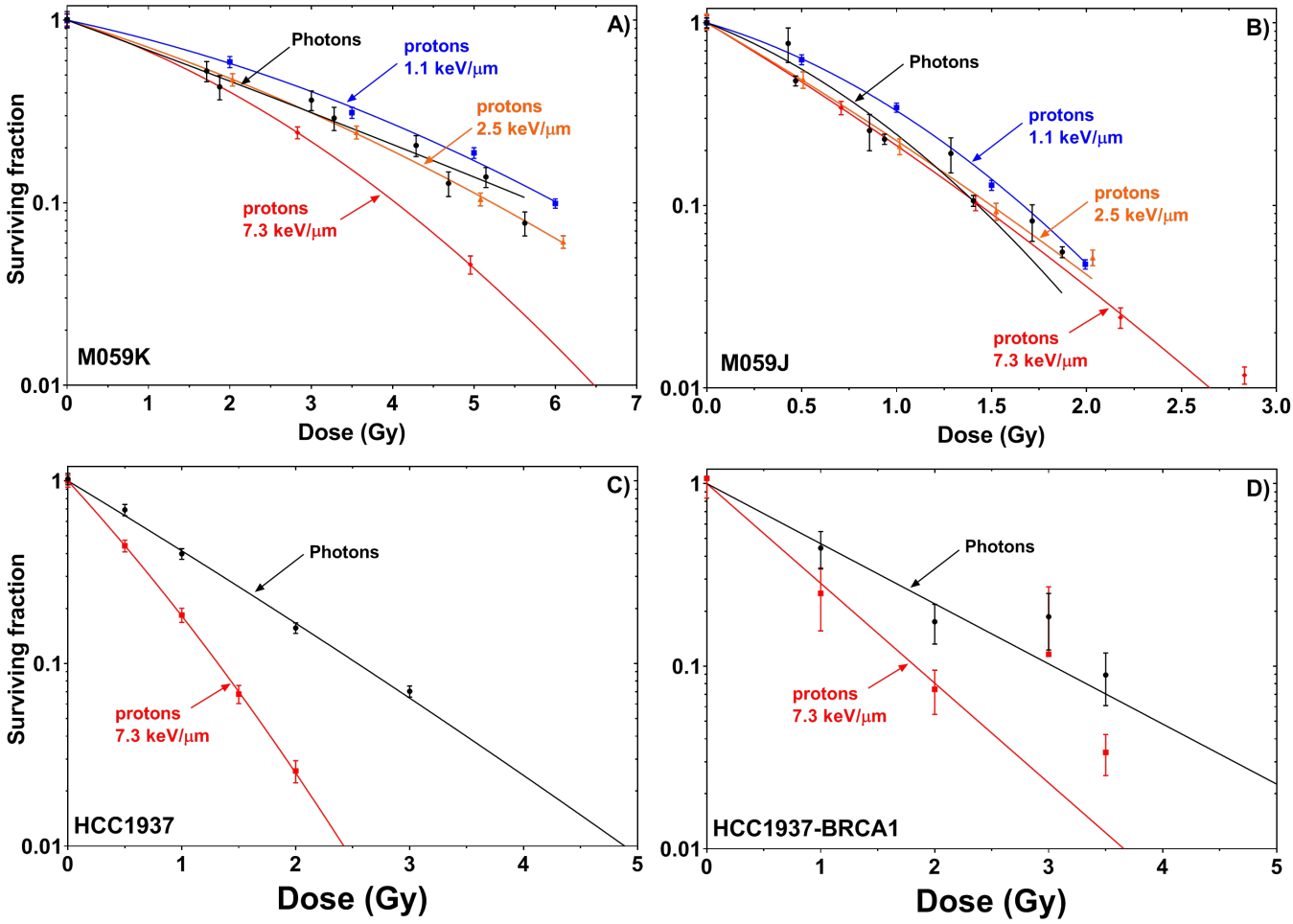
	SF_{2Gy}			
Cell Line	**Photon**	**1.1 keV/μm**	**2.5 keV/μm**	**7.3 keV/μm**
HT1080-shRAD51^{IND}	0.76 ± 0.03	0.773 ± 0.008	0.63 ± 0.02	0.60 ± 0.03
HT1080-shRAD51^{IND} + dox	0.33 ± 0.02	0.37 ± 0.02	0.36 ± 0.03	0.272 ± 0.006
HT1080-shDNA-PKcs	0.243 ± 0.007	0.186 ± 0.009	0.169 ± 0.008	0.149 ± 0.006
M059K	0.49 ± 0.03	0.58 ± 0.03	0.479 ± 0.008	0.405 ± 0.014
M059J	0.044 ± 0.004	0.0460 ± 0.0019	0.050 ± 0.004	0.039 ± 0.010
HCC1937	0.17 ± 0.03	—	—	0.025 ± 0.005
HCC1937-BRCA1	0.24 ± 0.06	—	—	0.11 ± 0.03

Supplementary Table 4. α and β values determined using the linear quadratic model. α and β values were determined using a nonlinear least squares fitting weighted by the inverse of the square of the standard deviation. We used GraphPad Prism (version 7.03, GraphPad Software, San Diego, CA).

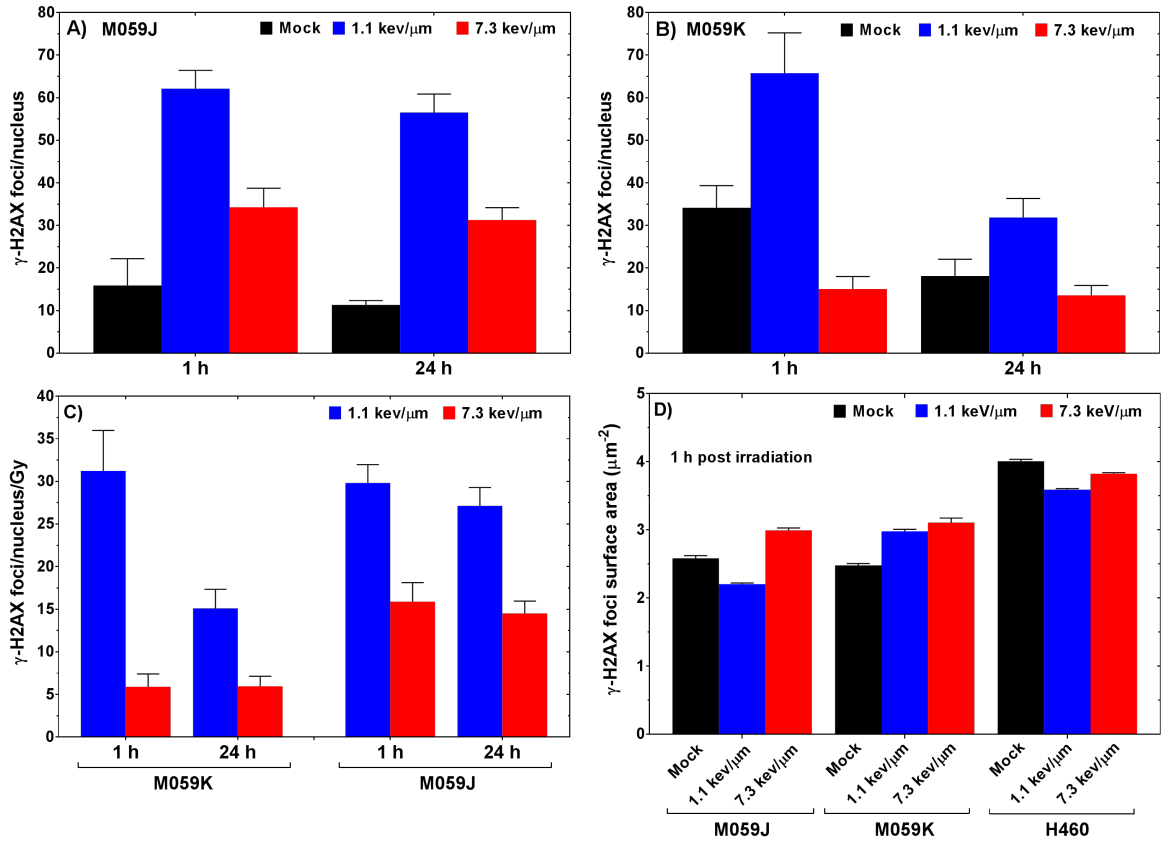
α (Gy^{-1})				
Cell Line	**Photon**	**1.1 keV/μm**	**2.5 keV/μm**	**7.3 keV/μm**
HT1080-shRAD51^{IND}	0.091 ± 0.03	0.051 ± 0.008	0.159 ± 0.03	0.078 ± 0.05
HT1080-shRAD51^{IND} + dox	0.544 ± 0.06	0.446 ± 0.04	0.415 ± 0.06	0.545 ± 0.02
HT1080-shDNA-PKcs	0.47 ± 0.04	0.69 ± 0.05	0.74 ± 0.05	0.90 ± 0.04
M059K	0.314 ± 0.06	0.222 ± 0.04	0.323 ± 0.012	0.333 ± 0.03
M059J	1.58 ± 0.05	0.69 ± 0.07	1.50 ± 0.09	1.62± 0.14
HCC1937	0.9 ± 0.09	-	-	1.57 ± 0.24
HCC1937-BRCA1	0.709 ± 0.1161	-	-	1.12 ± 0.119
β (Gy^{-2})				
HT1080-shRAD51^{IND}	0.024 ± 0.006	0.039 ± 0.002	0.036 ± 0.006	0.087 ± 0.010
HT1080-shRAD51^{IND} + dox	0.005 ± 0.014	0.025 ± 0.008	0.048 ± 0.011	0.053 ± 0.004
HT1080-shDNA-PKcs	0.12 ± 0.01	0.08 ± 0.02	0.07 ± 0.02	0.025 ± 0.009
M059K	0.021 ± 0.012	0.026 ± 0.007	0.023 ± 0.002	0.059 ± 0.005
M059J	0.00	0.42 ± 0.04	0.00 ± 0.03	0.00
HCC1937	0.000 ± 0.000	-	-	0.135 ± 0.15
HCC1937-BRCA1	0.000 ± 0.000	-	-	1.12 ± 0.000

Supplementary Table 5. Relative biological effectiveness (RBE) calculated for $D_{10\%}$. Uncertainties were calculated using error propagation theory.

	$RBE_{D10\%}$			
Cell Line	**Photon**	**1.1 keV/µm**	**2.5 keV/µm**	**7.3 keV/µm**
HT1080	1.00 ± 0.05	1.14 ± 0.04	1.31 ± 0.05	1.70 ± 0.07
HT1080-shRAD51^{IND}	1.00 ± 0.03	0.97 ± 0.03	1.06 ± 0.03	1.26 ± 0.03
HT1080-shDNA-PKcs	1.000 ± 0.014	1.10 ± 0.02	1.14 ± 0.02	1.19 ± 0.02
M059K	1.00 ± 0.04	0.90 ± 0.03	1.04 ± 0.03	1.34 ± 0.04
M059J	1.00 ± 0.04	0.89 ± 0.03	0.96 ± 0.07	1.07 ± 0.08
HCC1937	1.00 ± 0.14	-	-	2.00 ± 0.21
HCC1937-BRCA1	1.00 ± 0.23	-	-	1.65 ± 0.32



Supplementary Fig. 1. Clonogenic survival data for (A) M059K, (B) M059J, (C) HCC1937 and (D) HCC1937-BRCA1. Symbols represent the experimental data and lines represent fit to the linear quadratic model.



Supplementary Fig. 2. γ-H2AX radiation induced foci (RIF) at 1 h after irradiation with 1.1- or 7.3-keV/μm protons for M059K and M059J cell lines. Mock groups were subjected to the same conditions as the exposed groups aside from not being irradiated.

Supplemental Method 1. Reagents and cell culture conditions

M059K and M059J cells were cultured in Ham's F12 medium with L-glutamine adjusted to contain 15 mM HEPES, 0.5 mM sodium pyruvate, and 1.2 g/L sodium, and 10% fetal bovine serum (FBS). HCC1937, and HCC1937-BRCA1 cells were cultured in RPMI-1640 containing L-glutamine and supplemented with 10% FBS and 1% penicillin/streptomycin. All HT1080 cell lines were cultured in Dulbecco's minimal essential medium supplemented with 10% FBS and 1% penicillin/streptomycin.

All cell lines were maintained in a humidified atmosphere at 37ºC, 5% CO_2 in air, and were routinely subcultured before reaching 100% confluence by using 0.25% trypsin.

Reagent	Manufacturer	Part number
Dulbecco's Modified Eagle's Medium	Sigma	D6429
Ham's F12 Medium	Corning	10-080-CV
Minimum Essential Medium – Alpha	Corning	10-022-CV
RPMI-1640	Sigma	R8758
HEPES	Sigma	H0887
Trypsin 0.25%	Corning	25-053-CI
Penicillin Streptomycin solution	Hyclone	SV30010
Doxycycline	Sigma	D9891
Fetal Bovine Serum	Sigma	F0926
Phosphate buffered saline	Hyclone	SH30256
Mini-protean TGX precast protein gels	Bio-Rad	456-1095
Trans-blot turbo PVDF transfer pack	Bio-Rad	170-4156
SignalFire	Cell signalling Technology	6883
Tween 20	Sgma-Aldrich	P1379
Triton X-100	Fisher scientific	BP151-100
DMSO	Tocris	3716
Paraformaldehyde	Electron Microscopy Sciences	15710
Glycine	Sigma-Aldrich	G8898
Fish gelatin	Sigma-Aldrich	G7041
Goat serum	Abcam	AB7481
DAPI	Thermo-Fisher	62248
Non-fat dry milk	Bio-Rad	170-6404
γ-H2AX primary antibody	Millipore-Sigma	05-636
53BP1 primary antibody	Novus Biologicals	NB100-304
Actin primary antibody	Cell Signalling Technology	8457
Alexa Fluor 488 secondary antibody	ThermoFisher	A-11029
Alexa Fluor 594 secondary antibody	ThermoFisher	A-11037

Cell Line	Source	Culture Medium	FBS	L-glutamine	Penicillin / streptomycin, final concentration	Incubation time
M059K	ATCC	Ham's F12	10%	146	100/100	11–13 days
M059J	ATCC	Ham's F12	10%	146	100/100	11–13 days
HCC1937	ATCC	RPMI-1640	10%	300	100/100	18–20 days
HCC1937-BRCA1	Gift from Dr S. Stecklein (University of Kansas)	RPMI-1640	10%	300	100/100	18–20 days
HT1080	ATCC	MEM-Alpha	10%	292	100/100	7–9 days
HT1080-RAD51^{IND}	XXXX Lab (1)	MEM-Alpha	10%	292	100/100	7–9 days
HT1080-shDNA-PKcs	XXXX Lab	MEM-Alpha	10%	292	100/100	7–9 days

Units for L-glutamine are mg/L; for penicillin, U/mL; and for streptomycin, µg/mL.

References

1. XXXX.

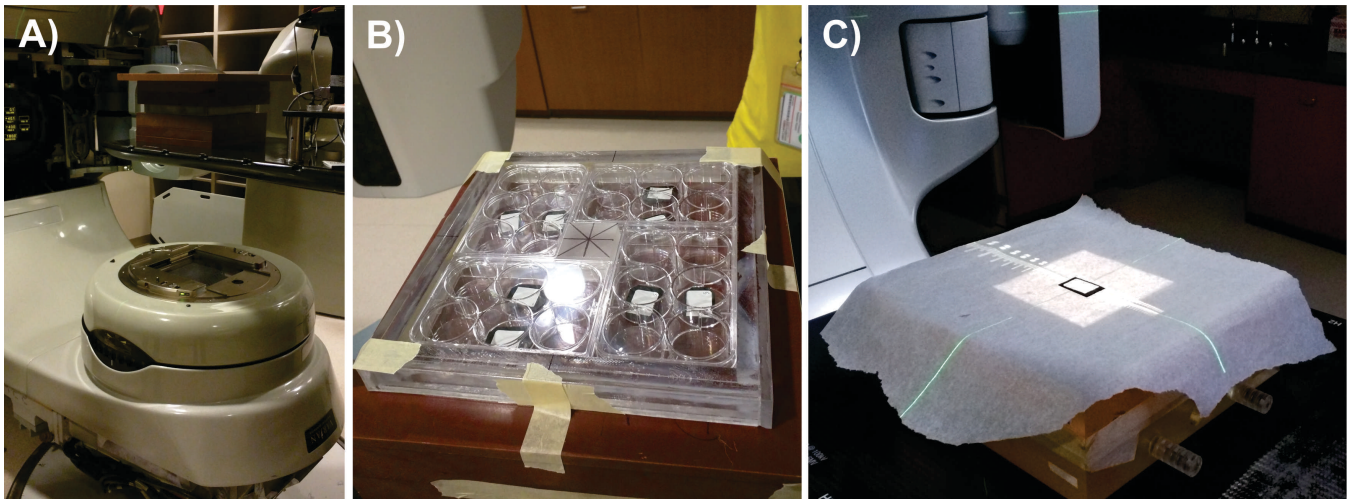
Supplemental Method 2. Photon dosimetry using optically stimulated luminescence dosimeters

For photon irradiations, cells were grown in 6-well plates and exposed to a flattened 6-MV x-ray beam from a clinical linear accelerator (linac) (Truebeam, Varian Medical Systems, Palo Alto, CA, USA) at a water equivalent depth of 10 cm under full lateral scatter and backscatter conditions. To provide the full scatter conditions, the 6-well plates were loaded into a custom plastic insert that fitted four 6-well plates and exposed at a gantry angle of 180º from underneath the treatment couch. Absorbed doses to water were measured inside of the wells of the 6-well plates, using custom cut optically stimulated luminescence detectors placed in the custom insert, in the conditions in which the cells were exposed. All photon irradiations involved the following beam setup conditions:

Facility: XXXX
Machine: Truebeam 2
Field size: 40 cm × 40 cm at isocenter plane
Energy: 6 MV
Dose rate: 600 MU/min
Mode: flattened beam
Gantry angle: 180º (beam through the couch top)
Source to surface distance (SSD): isocenter plane set to bottom of couch
Water equivalent depth: 10 cm

Measurements were done using 6-mm diameter custom-cut Al_2O_3:C OSLDs, which were read out in an in-house built OSLD reader as described previously (1,2). The OSLDs (packaged in black electrical tape) were calibrated by placing them at a depth of 1.5 cm in solid water along the beam's central axis with 10 cm of backscatter and using a 100 cm source-to-surface distance (SSD) setup. They were then exposed to doses of ranging from 0.3 to 0.5 Gy and later read out to calibrate the OSL signal intensity to absorbed dose which is linear in this range of doses.

OSLDs from the same batch were then placed in the wells of empty 6-well plates and then inserted into a custom acrylic insert (Supplementary Fig. 3) that was built to minimize air gaps and provide support for additional backscatter material used in the cell irradiations. We considered the water equivalent thickness of the patient couch (0.7 cm) as well as the water equivalent thickness of the insert to determine the 10 cm total water equivalent thickness of the irradiation conditions. The OSLDs were then exposed to 50 monitor units (MU) in the same conditions as the cell irradiations – at a depth of 10 cm in solid water with 10 cm of backscatter material. After reading out the detectors irradiated at the cells' position, they were compared to the calibration detectors to determine the absorbed dose they received from 50 MU of machine output. This was then used to calculate a dose/MU calibration factor, $f_{Dose/MU}$ = 0.00689 ± 0.00003 Gy/MU for cells irradiated under the same conditions. We then calculated the dose the cells received in terms of the number of MU they were exposed to as $Dose = MU \cdot f_{Dose/MU}$.



Supplementary Fig. 3. (A) Photon beam setup. Cells reside in 6-well plates placed in a custom plastic insert, visible as the clear section in the middle of the brown solid water. (B) OSLDs packaged in black electrical tape were placed in the wells of 6-well plates placed in a custom acrylic insert. (C) OSLDs packaged in black electrical tape were placed at a depth of 1.5 cm in solid water using a 100 cm SSD setup with 10 cm of backscatter. The 1.5 cm solid water above the OSLDs is omitted so that the OSLDs may be visualized.

References

1. XXXX.
2. XXXX.

Supplemental Method 3. Proton beam dosimetry using ionization chambers

Proton irradiations were done at the XXXX. Cells were exposed to a passive scattered 100-MeV unmodulated proton beam. The beam was delivered from bottom to top at 180º gantry angle with a fully retracted medium snout with a field size of 18 cm × 18 cm at the isocenter plane. Cells were irradiated through the treatment couch at three water equivalent depths (1.21, 3.91, and 4.42 cm) to generate three distinct fluence-weighted LET values (1.1, 2.5, and 7.3 keV/μm). The LET values were obtained by using a validated Monte Carlo model of the beam line (Supplemental Method 4). Absorbed dose to water measurements were done with a calibrated parallel plate ionization chamber (34045, Advanced Markus Chamber, PTW-Freiburg, Freiburg, Germany). To obtain dose measurements under the conditions in which cells were exposed, we cut the bottom of the 6-well plates and removed the protective cap of the chamber to simulate the water equivalence thickness in which the cells adhered in the bottom of the wells. This setup was also used to obtain the water equivalent depth of each LET condition (1.21, 3.91 and 4.42 cm), which in turn was used as input to obtain the depths for the Monte Carlo LET calculations (Supplemental Method 4). All proton irradiations involved the following beam setup conditions:

Facility: XXXX
Gantry: 2
Snout: medium
Snout position: 45 cm (fully retracted)
Field size: 18 cm × 18 cm at isocenter
Aperture: 18 cm × 18 cm
Energy: 100 MeV
Range: 4.3 cm
Mode: pristine un-modulated beam
Range modulator wheel (RMW): 15 parked on scatter foil
Gantry angle: 180º (beam through the couch top)
Couch coordinates: 0 cm, -20 cm, 15.2 cm
Source to surface distance (SSD): isocenter plane set to bottom of couch
Couch top: large couch

Measurements were performed using a calibrated Advanced Markus ionization chamber (PTW, TN34045, S/N 0300) without its protective cap to established water equivalent thicknesses (WETs) that were as close as possible to the conditions in which the cells where exposed. The chamber was placed in a chamber holder and measurements were made using different WETs, which were obtained using water equivalent plastic (457-CTI, Gammex, Inc., Middleton, WI, USA). Measurements were done for WET of 1.1 cm, which is the WET of the couch and from 3.5 cm to 4.5 cm in steps of 1 mm.

For the clonogenic cell survival experiments, we placed the dishes on the couch to avoid media from spilling. In this setup the beam traversed the couch, water equivalent plastic slabs and the bottom of the dish

before reach the adherent cells. To determine the dose output in the same conditions in which the cell survival irradiations were performed, we cut the bottom of 6-well plates, T12.5, T25 and T75 flasks to include them as additional material in front of the ionization chamber (Supplementary Fig. 4). Model numbers of the plates and flasks in which WET was measured are in Supplementary Table 6. The results of the dose output for the different conditions and WET of the plate and flasks are in

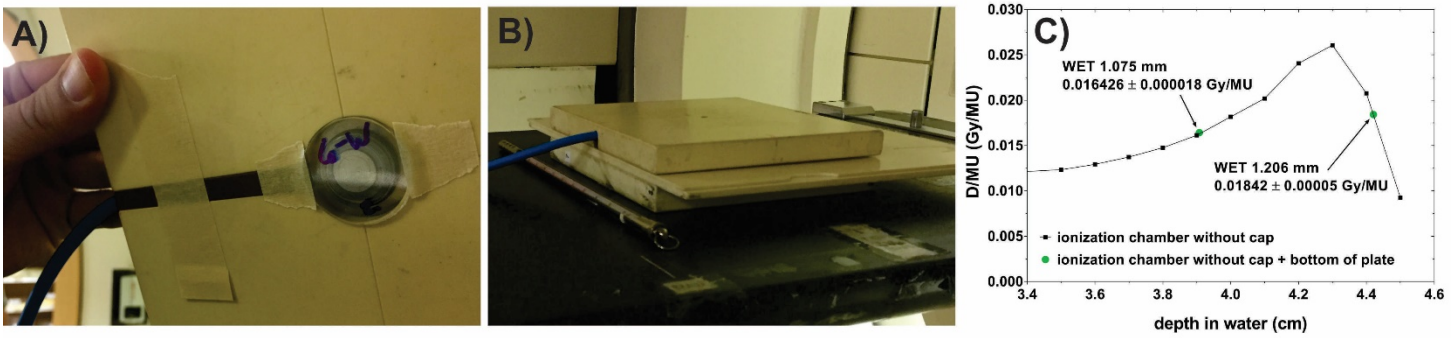
Supplementary Table 7 and Supplementary Table 8. Throughout this work we only used the 6-well plate. Thus, the total water equivalent depth for each condition we used in this work was 1.21, 3.91 and 4.42 cm (Supplementary Table 8). We did not measure the WET of the dishes for the 1.1 cm condition. Instead we assumed that the WET was the same as for the 3.8 cm condition.

Supplementary Table 6. Manufacturers and model numbers of the dishes used in the irradiations.

	Material	**Manufacturer**	**Model**	**Part number**
6-well plate	Polystyrene	Corning Inc	Costar	3506
T12.5	Polystyrene	Celltreat	12.5 cm^2 Tissue Culture Flask	229321
T25	Polystyrene	Thermo Scientific	Nunc EasYFlask 25 cm^2	156367
T75	Polystyrene	Thermo Scientific	Nunc EasYFlask 75 cm^2	156499

Supplementary Table 7. Dose output and WET of bottom of dishes used in irradiations for clonogenic cell survival experiments. 1.1, 3.8 and 4.3 cm correspond to the water equivalent depth of the couch plus plastic slabs that were placed in front of the chamber with the dish bottom. WET (mm) correspond to the measured WET of the material for the dishes that were placed in front of the chamber at the specified water equivalent depths.

	D, Gy/MU			**WET, mm**	
	1.1 cm	**3.8 cm**	**4.3 cm**	**3.8 cm**	**4.3 cm**
None	0.00790 ± 0.00002	0.014762 ± 0.000040	0.026062 ± 0.000014	—	—
6 well	—	0.016426 ± 0.000018	0.018424 ± 0.000055	1.075	1.206
T12.5	—	0.016737 ± 0.000014	0.016658 ± 0.000021	1.245	1.359
T25	—	0.016604 ± 0.000013	0.017498 ± 0.000040	1.175	1.286
T75	—	0.017061 ± 0.000036	0.014576 ± 0.000033	1.420	1.540



Supplementary Fig. 4. (A-B) Irradiation setup to determine dose output for the clonogenic cell survival irradiations. The chamber was secured in a chamber holder, a cutoff of a dich bottom were secured in front of the chamber (A) and chamber holder with chamber and dish cutoff were place behind water equivalent plastic slabs, which were placed on the patient couch (B). Note that in this setup the beam traversed the couch, water equivalent plastic and dish cutoff before reaching the sensitive volume of the chamber. (C) Ionization chamber measurements as a function of water equivalent depth. Green circles represent the output measured with the bottom of the plate in front of the ionization chamber for water equivalent depths of 3.8 and 4.3 cm.

Supplementary Table 8. Total water equivalent depth that accounts for the WET of the bottom of the dishes for each conditions used for the irradiations.

Water equivalent depth (cm)				
None	6 well	T12.5	T25	T75
1.1	1.21	1.22	1.22	1.24
3.8	3.91	3.92	3.92	3.93
4.3	4.42	4.44	4.43	4.45

All the experiments performed in this work were done in gantry 2 at the XXXX. Because the beam traversed the couch, we used the same couch top and the same couch position to minimize variabilities due to heterogeneous couch density.

Supplemental Method 4. Monte Carlo simulations

Monte Carlo simulations were performed to obtain the LET information under the conditions in which the cells were exposed. The simulations were performed using MCNPX version 2.7 (1). The proton beam was simulated in a validated model of the double scattering proton beam line of the XXXX (2). A water phantom was modelled to be located with the upstream surface located at isocenter, 273 cm from the particle source. The snout of the beam line was at the maximum distance of 45 cm. In the water phantom, a series of 50 disks, each 1 mm in thickness and with a diameter of 0.5 cm served as tallies for the proton fluence. The MC tallies were shifted by 0.2 mm to match the experimental data. The fluence was scored with an energy resolution of 100 keV. Additionally, the energy deposition was scored with a circular mesh tally of similar radius but with a resolution in depth of 0.1 mm. The transport of the protons was terminated at a cutoff threshold of 1 keV, and a total of 2×10^8 source particles were transported to ensure statistical uncertainties of less than 2% at dose locations larger than 5% of the maximum dose.

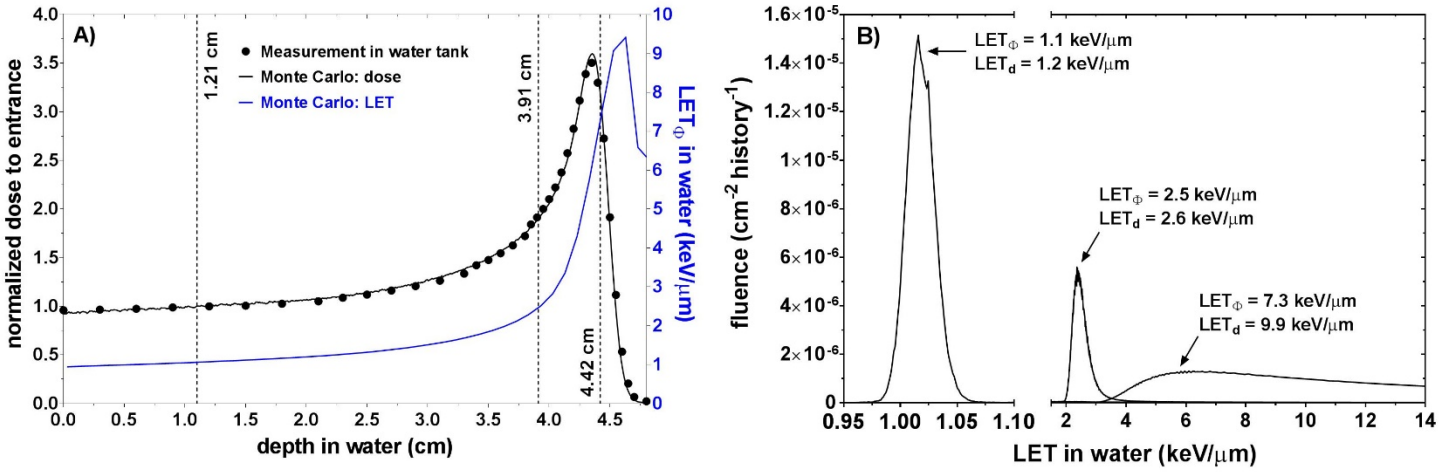
The fluence-weighted LET_Φ and the dose-weighted LET_d were calculated as (3-5):

$$LET_\Phi = \frac{\int_0^\infty S_{el}(E)\Phi(E,z)\mathrm{d}E}{\int_0^\infty \Phi(E,z)\mathrm{d}E} \qquad \text{eq. 1}$$

and

$$LET_d = \frac{\int_0^\infty S_{el}^2(E)\,\Phi(E,z)\mathrm{d}E}{\int_0^\infty \Phi(E,z)\,\mathrm{S(E)}\mathrm{d}E}, \qquad \text{eq. 2}$$

where S_{el} is the electronic stopping power of the protons with energy E and the fluence $\Phi(E,z)$ at the location z. To define the radiation conditions, we used the fluence-weighted LET_Φ because it has been shown to be less dependent on parameters of the simulation (5) and therefore more robust than dose-weighted LET_d (Supplementary Fig. 5).



Supplementary Fig. 5. Monte Carlo simulations of the setup used in the experiments. (A) Normalized depth dose distribution to demonstrate that the simulated dose agrees with measured dose using multi-layer ionization chamber device (Zebra, IBA Dosimetry GmbH, Germany). (B) Electronic stopping power S_{el} spectra at the positions in which cells were exposed. The value of the electronic stopping power is approximately equal to the value of the LET for the energy

range under investigation. The values of the fluence- (LET_f) and dose-weighted LET (LET_d) as calculated using eqs. 1 and 2, respectively, are presented in the plot.

References

1. Waters LS, Hendricks J, McKinney G, Mcnpx user's manual version 2.4.0. Los Alamos: Los Alamos National Laboratory, 2004.
2. XXXX.
3. Guan F, Peeler C, Bronk L, et al. Analysis of the track- and dose-averaged let and let spectra in proton therapy using the geant4 monte carlo code. *Med Phys* 2015;42:6234.
4. Wilkens JJ, Oelfke U. A phenomenological model for the relative biological effectiveness in therapeutic proton beams. *Phys Med Biol* 2004;49:2811-2825.
5. Granville DA, Sawakuchi GO. Comparison of linear energy transfer scoring techniques in monte carlo simulations of proton beams. *Phys Med Biol* 2015;60:N283-N291.

Supplemental Method 5. Clonogenic cell survival and colony-counting with ImageJ macros

Cells were trypsinized 20–24 h before irradiation and seeded into 6-well plates at appropriate numbers for each dose. Plates were then irradiated to at least five dose levels ranging from 0 to 9 Gy. Each cell line was plated in triplicate or sextuplicate and a minimum of three independent experiments were done. HT1080 cells were incubated for 8-9 days after radiation exposure. M059K and M059J cells were incubated for 11-12 days. Both HCC1937 cell lines were incubated for 18-20 days. All radiation treatments were done at room temperature. All control conditions followed the same experimental process minus the radiation exposure.

After the designated time, cells were fixed and stained with 70% ethanol and 2% crystal violet. Plates were allowed to air-dry overnight before undergoing high-resolution scanning (Expression 10000 XL, Epson).

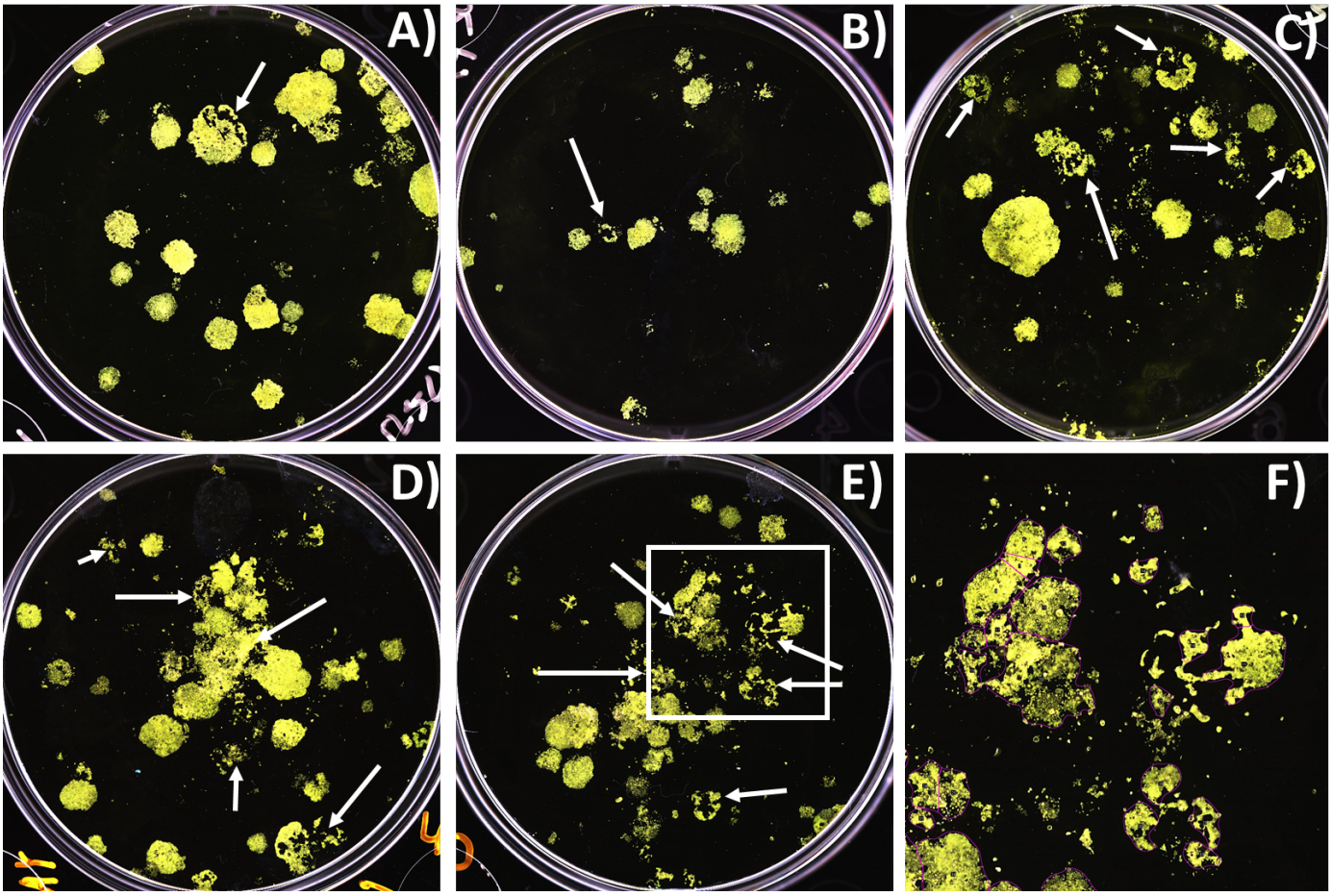
Colonies were scored by using custom ImageJ macros that were individually optimized to score the colonies in the cell lines used in this work. Briefly, for each cell line, we calibrated brightness and cell density thresholds which we used to exclude noise and small groups of senescent cells via Gaussian blurring and intensity thresholding. We then segmented the colonies in the images and, using the smaller colonies, we calibrated the area of the minimum colony forming unit by comparing the segmented colony areas to the number of cells in the segmented colonies counted under bright field microscopy. From these measurements, we calculated the pixel area corresponding to a 50 cell colony for each cell line, and used these pixel area thresholds to score all the colonies containing 50 or more cells.

Data were first analyzed within each independent experiment. For each experimental replicate that used multiple seeding numbers, z-tests were used to eliminate any systematic undercounting errors of wells with higher seeding numbers. Data from higher-seeded wells were excluded if they were more than two standard deviations lower than the data from the lower-seeded wells. For each experiment, we determined the plating efficiency first by computing the survival quotient for each dose, $SQ_D = N_{colonies,D} / N_{seeded,D}$. Then the SQ_D values were plotted versus dose and fit via variance weighted non-linear least squares minimization to an unnormalized version of the linear quadratic model: $SQ = PE \exp(-\alpha D - \beta D^2)$, where PE is the plating efficiency and α and β are free parameters. The plating efficiency determined from each experiment's fit was then used to calculate the survival fraction for each dose, $SF_D = SQ_D / PE$. The survival fractions calculated across all independent experiments were then combined by calculating the variance-weighted survival fraction for each dose, $\overline{SF_D}$. The $\overline{SF_D}$ values were then plotted versus dose and fit via variance-weighted non-linear least squares minimization to the linear quadratic survival model $SF = \exp(-\alpha D - \beta D^2)$. We then quantified these survival curves by calculating the dose at which the survival fraction was 10%, that is, $D_{10\%}$. The uncertainty in $D_{10\%}$ was estimated using error propagation theory. Statistical significance to compare $D_{10\%}$ and sensitization values among different conditions were done using the t-test.

To assess response within and between cell lines, we used the sensitization relative to wild-type photon response, defined as $D_{10\%, photon\text{-}WT} / D_{10\%, L}$, where the subscript L indicates the LET of the radiation. This metric quantifies the sensitivity of a given radiation type L and/or genotype relative to the response of the wild-type (WT) to photons. We also calculated the sensitization enhancement ratio, defined as $D_{10\%, WT, L} / D_{10\%, Mut, L}$. In

this case, the radiation type L is kept constant, and the sensitivity for a given radiation type and condition is quantified purely as a result of the genotype. When normalized data were statistically compared, uncertainty on the control value of unity was assumed to be 0. However, the uncertainty of the denominator was propagated to the non-control normalized data.

HCC1937-BRCA1 data was quantified using the same method as described previously. However, it was perceived that at higher doses colony morphology was altered to an extent that was incompatible with the imageJ macro. In this scenario the macro was run as described previously and each image was checked manually. For HCC1937-BRCA1, high dose groups were excluded from all analysis due to the inability to count colonies accurately (Supplementary Fig. 6).



Supplementary Fig. 6. Clonogenic assay colonies from HCC1937-BRCA1 cells scanned with a high-resolution Epson expression 10000 XL film scanner. White arrows highlight areas that are representative of regions difficult to count. (A,B) Examples of control and low dose experiments, where colonies showed simple circular structure. These were generally well contoured by the imageJ macro. (C-E) Examples of “harder to count” regions/colonies that were poorly contoured, these regions occurred more frequently in higher dose groups. (F) Magnified from the square highlighted in (E) shows outlined colonies generated by the imageJ macro.

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Supplemental Method 6. Immunohistochemical analyses

Cell lines were fixed in 4% paraformaldehyde for 10 min at room temperature at 1 h and 24 h after a 2-Gy irradiation with protons. All radiation treatments were done at room temperature. All control conditions followed the same experimental process minus the radiation exposure. Cells were then washed three times with PBS for 5 min each time at room temperature and then then permeabilized with 0.2% Triton-X for 10 min at room temperature, followed by blocking with 0.2% fish gelatin and 5% goat serum (this combination of blocking agents yielded the cleanest background for imaging). Cells were then stained with primary antibodies (γ-H2AX 1:5000) (JWB301, EMD Millipore, Burlington, MA, USA) overnight at 4°C. After primary antibody staining, samples were washed with PBS three times for 10 min per wash with gentle agitation. Samples were then incubated with Alexa Fluor 488 (#A-11029, Thermo Fisher Scientific, Waltham, MA, USA) for 1 h at room temperature with gentle agitation. Cells were washed with PBS three times, 10 min per wash. Nuclei were then stained with 4′,6-diamidino-2-phenylindole (5 µg/mL) (#62248, Thermo Fisher Scientific) for 10 min at room temperature. Samples were washed with PBS twice for 5 min per wash. Cells were mounted with Fluromount-G (#100-01, SouthernBiotech, Birmingham, AL, USA) and sealed with nail polish. Images were taken as confocal z-stacks (7–15 µm in depth, 0.44-µm step size) from a minimum of 4 random fields with a confocal scanning microscope (FluoView 1200/IX-83, Olympus America Inc., Center Valley, PA, USA).

Foci and nuclei were detected and analyzed in Imaris (Bitplane AG, Zurich, Switzerland) using the Imaris Cell module. Imaris Cell can automatically detect cell bodies, nuclei and vesicles, and in this case the DAPI stained nucleus was treated as the cell body and the foci were treated as vesicles. For consistency, the algorithm was trained on the 4.3 cm depth, 2 Gy dose, 1 h time-point condition for each cell line. Nuclear surfaces were detected using 1.0 µm surface detail and a manually selected Quality threshold before performing 3-D watershedding using a seed diameter of 8-10 µm (cell-line dependent) to separate adjacent nuclei. Finally, a volume threshold was applied of approximately half the number of voxels in the average nucleus to exclude nuclei which were clipped by the edge of the field.

Vesicle channels were created for the γ-H2AX foci spots. The initial guess for the spot diameter was 0.5 µm and variable spot sizes (region-growing) were enabled. Spots were spherical and did not account for the confocal z-axis asymmetry. Manual Quality thresholds were selected for each protein and cell line, but they ended up being similar across all cases. Parameters such as the Spot Diameter, Spot Mean Intensity, Spot Area, Spot Volume, Spot Location, Nuclei Volume, and Number of Spots per Nucleus were flagged for output. Creation parameters were saved for each cell line and all images were batch processed using these parameters and the Imaris Batch module.

We used γ-H2AX foci as a surrogate for DSBs. Raw numbers of RIF for each exposure condition was subtracted by the background of endogenous foci, which was determined from mock irradiated groups at 1 h and 24 h. RIF data were normalized to the proton fluence (units μm^{-2}) to allow comparisons of the efficiency of RIF production and recovery.

Supplemental Method 7: Western blotting

Whole cell lysates were prepared from HT1080-RAD51^{IND} cells that had been treated with doxycycline for 48 h with and without a 24-h recovery, or a 72-h treatment. A western blotting kit (#12957, Cell Signaling Technology, Danvers, MA, USA) was used as follows. Protein was extracted from whole cell lysates and quantified; 20 µg of protein was heated to 95°C for 5 min and immediately cooled on ice. Samples were spun at 3,500 × *g* for 4 min at 4°C, and the supernatant was removed and combined with a suitable volume of loading buffer. Each sample was resolved on a 4%-20% gradient SDS-PAGE gel (#456-1095, Bio-Rad, Hercules, CA, USA) run at 100 V for 15 min followed by 1 h at 160 V (PowerPac HC, Bio-Rad). Proteins were then transferred to nitrocellulose membranes with the (Trans-Blot Turbo transfer system (Bio-Rad). Membranes were blocked in blocking buffer before being incubated overnight at 4°C with primary antibodies for RAD51 (Ab88752: 1 µg/mL, Abcam, Cambridge, UK) and actin (Ab8224, 1 µg/mL, Abcam). The blots were washed in TBST (mixture of tris-buffered saline and polysorbate 20) before incubation with species conjugated with horseradish peroxidase secondary antibody. Blots were then incubated with a chemiluminescent substrate (#6883, SignalFire ECL Reagent, Cell Signaling Technology) for 1 min at room temperature before being imaged (ChemiDoc XRS+, Bio-Rad).