Data S1

Custom macro to count colonies from the clonogenic assay using ImageJ (Fiji) software.

```
setTool("oval");
makeOval(1038, 24, 51, 1473); /// This forms an oval region to select the well of the plate.
makeOval(1038, 24, 777, 1473);
makeOval(321, 24, 1494, 1473);
waitForUser("Select well region."); /// ImageJ waits for the user to verify whether the well of the plate is correctly selected.
setBackgroundColor(0, 0, 0);
run("Clear Outside");
run("8-bit");
run("Threshold...");
waitForUser("Set your threshold"); /// ImageJ waits for the user to adjust the threshold.
setOption("BlackBackground", true);
run("Convert to Mask");
run("Analyze Particles..."); /// Set the minimum and maximum size of the counted objects: in the non-irradiated control, manually identify a colony containing 50 cells under the microscope. The area of this colony (in pixels^2) will be used in this step as the minimum size of particles to be counted. Therefore, only colonies containing 50 or more cells will be included in the count.
waitForUser("Save your results of cell couting"); /// ImageJ waits for the user to save the results.
close("*"); /// This closes all images.
close("Results"); /// This closes the Results window.
close("Threshold"); /// This closes the Threshold window.
close("Summary") /// This closes the Summary window.
```

Data S2

Complete step-by-step guide for the nonlinear fits during the analysis of the cell survival curves using GraphPad Prism software (version 9).

After following the previous steps, a nonlinear regression is used to determine the plating efficiency (PE) in *Analyze* → *Nonlinear fit*. In the non-linear regression parameters, 'explicit equation' was selected as the equation type and defined the equation as . Initial values follow specific rules (1 as the initial value to be fit for PE; 1 and 0.1 as/(Value of X at YMid) for A and B, respectively), and the default range starts graphing the curve at the smallest X value, with no default constrains applied. In the methods configurations for the nonlinear regression, outliers should be reported, and the fitting method should be 'least squares regression', with a medium convergence criterion, maximum 1,000 iterations, automatically switching to strict when needed. No weighting method is applied. N and scatter among replicates (from the SD or SEM) are considered. In the 'Compare' menu, no comparisons are selected. No constrains are used. In the 'Range' menu, both minimum and maximum X values are chosen automatically. In the 'Confidence' menu, the confidence intervals of parameters should be calculated, and unstable parameters and ambiguous fits should be identified. Goodness-of-fit is also quantified. After pressing *OK*, PE values were determined.

After determining the PE and following subsequent steps, the survival fraction results are fit with the linear-quadratic model of radiobiology in *Analyze* → *Nonlinear fit* with the equation. Initial values follow specific rules (0.1 as the initial value to be fit, for both A and B), and the default range starts graphing the curve at the smallest X value. Both A and B must be greater than zero and the ratio A/B should be reported ('Transforms to report' menu, in which P1=A and P2=B). In the 'Method' menu, outliers should be reported, and the fitting method should be 'least squares regression', with a medium convergence criterion, maximum 1,000 iterations, automatically switching to strict when needed, and with weighting by 1/SD^2. In the 'Compare' menu, no comparisons are selected. In the constraints, A must be >0. In the 'Range' menu, both minimum and maximum X values are chosen automatically. In the confidence menu, the CI of parameters and goodness-of-fit should be calculated as previously. The α/β values are determined after pressing *OK*, both for the entire dataset and for each independent experiment separately.
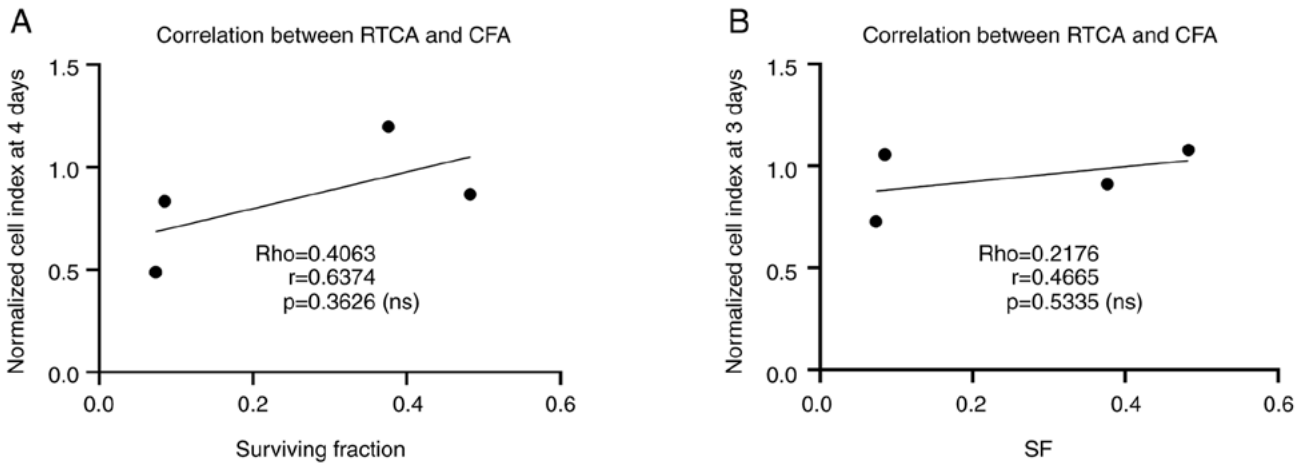
Figure S1. Correlation between surviving fractions and cell index values in irradiated assays. (A) Cell index values 4 days after plating and surviving fractions at the end of the experiment (6 days post-plating). (B) Cell index values 3 days after plating and surviving fractions at the end of the experiment (6 days post-plating). RTCA, real-time proliferation assay. CFA, colony formation assay.