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Supplemental Information

RAD51 Is a Selective DNA Repair Target to Radiosensitize Glioma Stem Cells

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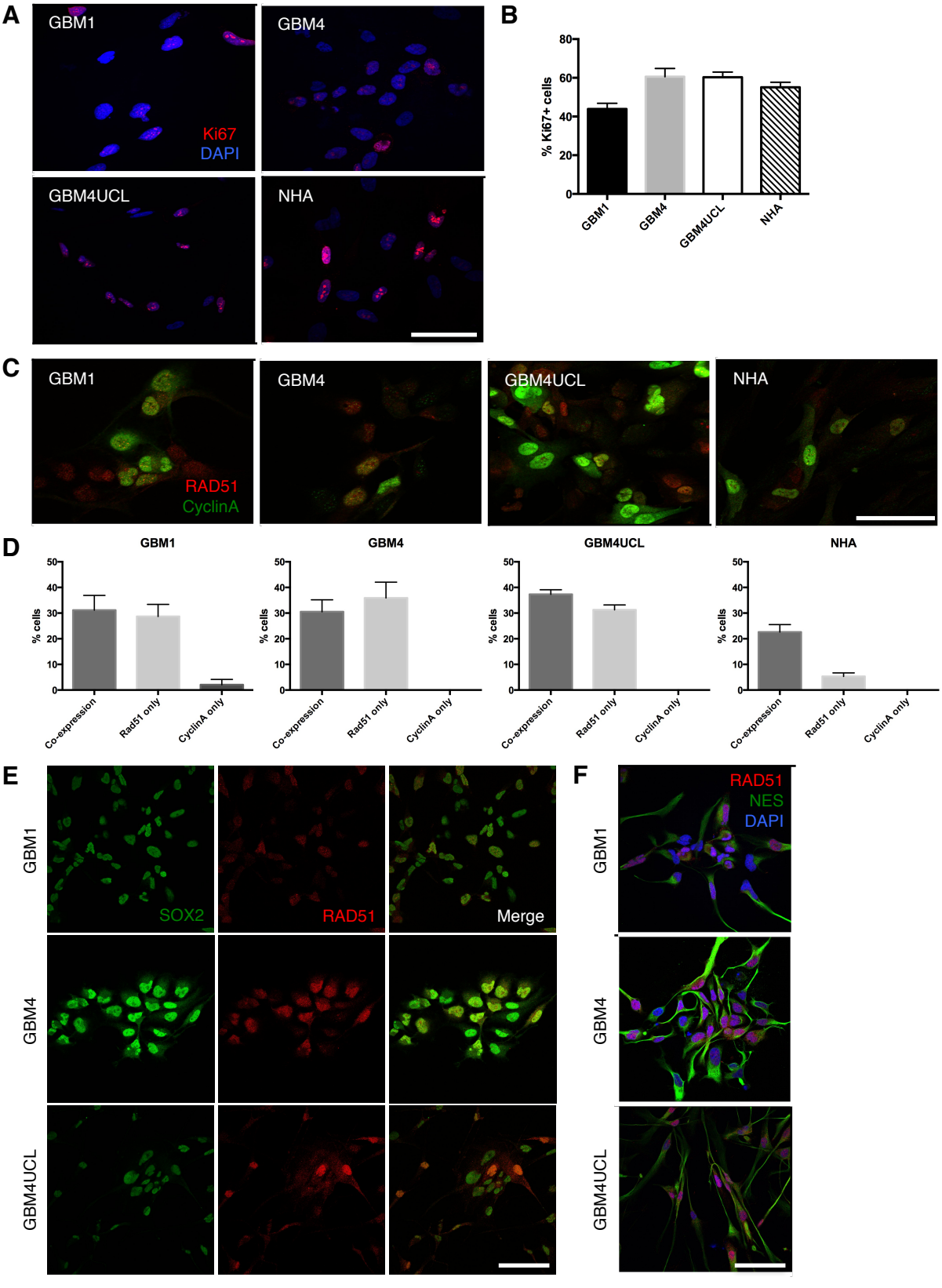

A
GBM1
Ki67
DAPI
GBM4
GBM4UCL
NHA
B
% Ki67+ cells
80
60
40
20
0
GBM1
GBM4
GBM4UCL
NHA
C
GBM1
RAD51
CyclinA
GBM4
GBM4UCL
NHA
D
GBM1
GBM4
GBM4UCL
NHA
% cells
50
40
30
20
10
0
Co-expression
Rad51 only
CyclinA only
E
GBM1
GBM4
GBM4UCL
SOX2
RAD51
Merge
F
RAD51
NES
DAPI
GBM1
GBM4
GBM4UCL

Figure S1, related to Figure 1. (A) GSC and NHA grown in standard conditions were stained for the proliferation marker Ki67. **(B)** The percentage of proliferative (Ki67 +ve) cells in three GSC lines and NHA (n=4 independent experiments). **(C)** Cyclin A (green) and RAD51 (red) were visualised by immunofluorescence (IF) microscopy in GSC to determine the overlap between RAD51 and cells in the S/G2 phase of the cell cycle. **(D)** Quantification of the number of cells co-expressing RAD51 and CyclinA, or expressing either protein individually for each of the cell lines shown in **(C)** (n=4 independent experiments). (**E)** Dual staining for RAD51 and SOX2 proteins visualised by immunofluorescence to confirm co-localisation identified in single cell PCR data. (**F)** Co-expression of RAD51 and an additional stem cell marker NES determined by IF in each of the three GSC lines used in this study. In all panels, scale bars represent 20 μm

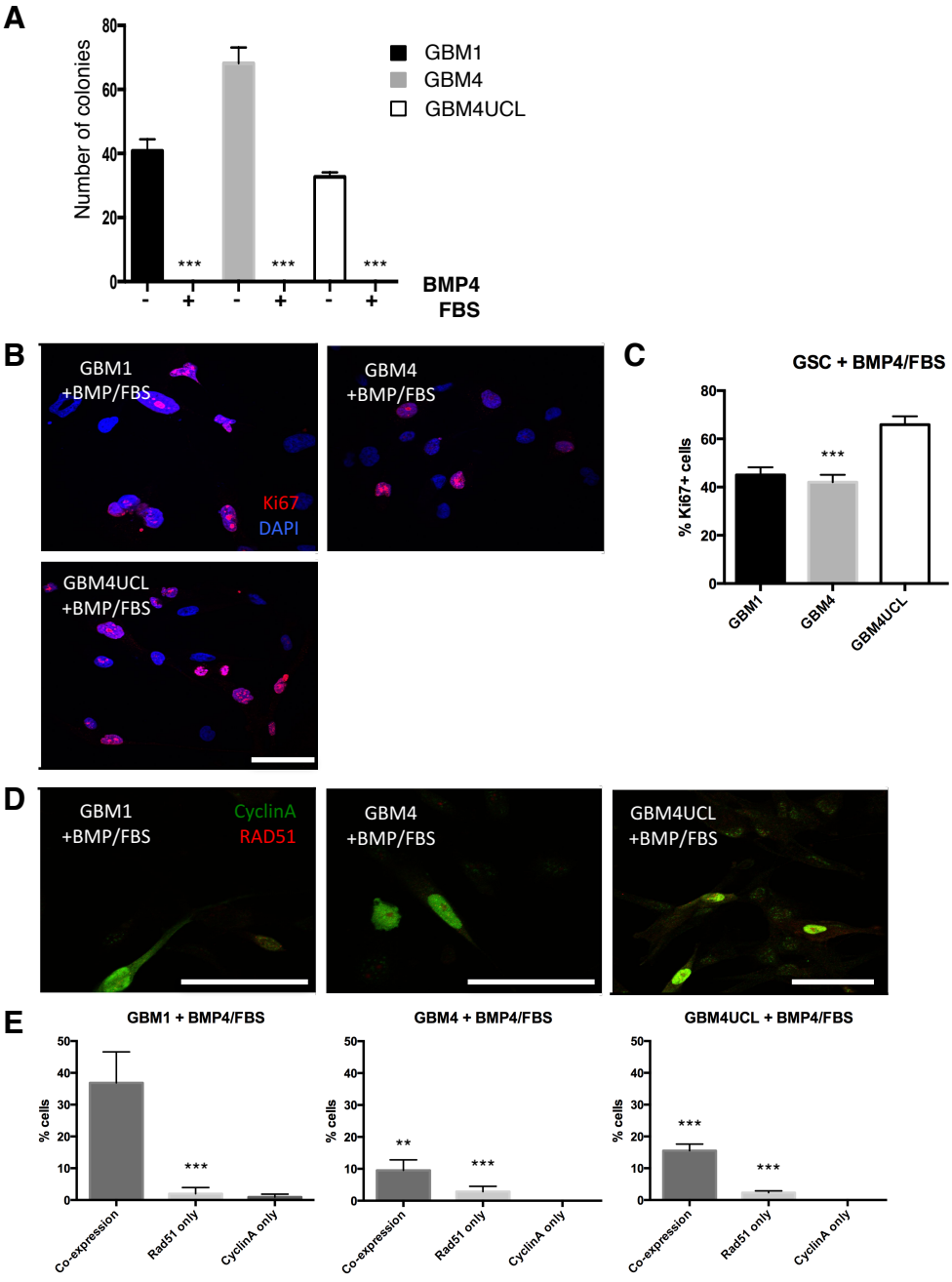


Figure S2, related to Figure 2. (A) Effect of treatment with BMP4 and FBS on the ability of GSC to form colonies in a clonogenic assay (n=3 independent experiments). **(B)** GSC grown in NB-BMP4-FBS (treated) were stained for the proliferation marker Ki67. **(C)** The percentage of proliferative (Ki67 positive) cells in three treated GSC lines (n=4 independent experiments). Statistical significance was determined relative to the corresponding undifferentiated cells (Figure S1B).**(D)** RAD51 (red) and Cyclin A (green) expression determined by IF in GSC grown in NB-BMP4-FBS. **(E)** Quantification of the number of cells co-expressing RAD51 and Cyclin A, or expressing either protein individually for each of the differentiated GSC lines shown in **(D)** (n=4 independent experiments). Significant differences were assessed relative to the equivalent populations in undifferentiated cells (Figure S1D). Throughout the figure: error bars = SEM, statistical significance was calculated using one-way ANOVA, ** p=<0.05, *** p=<0.001.

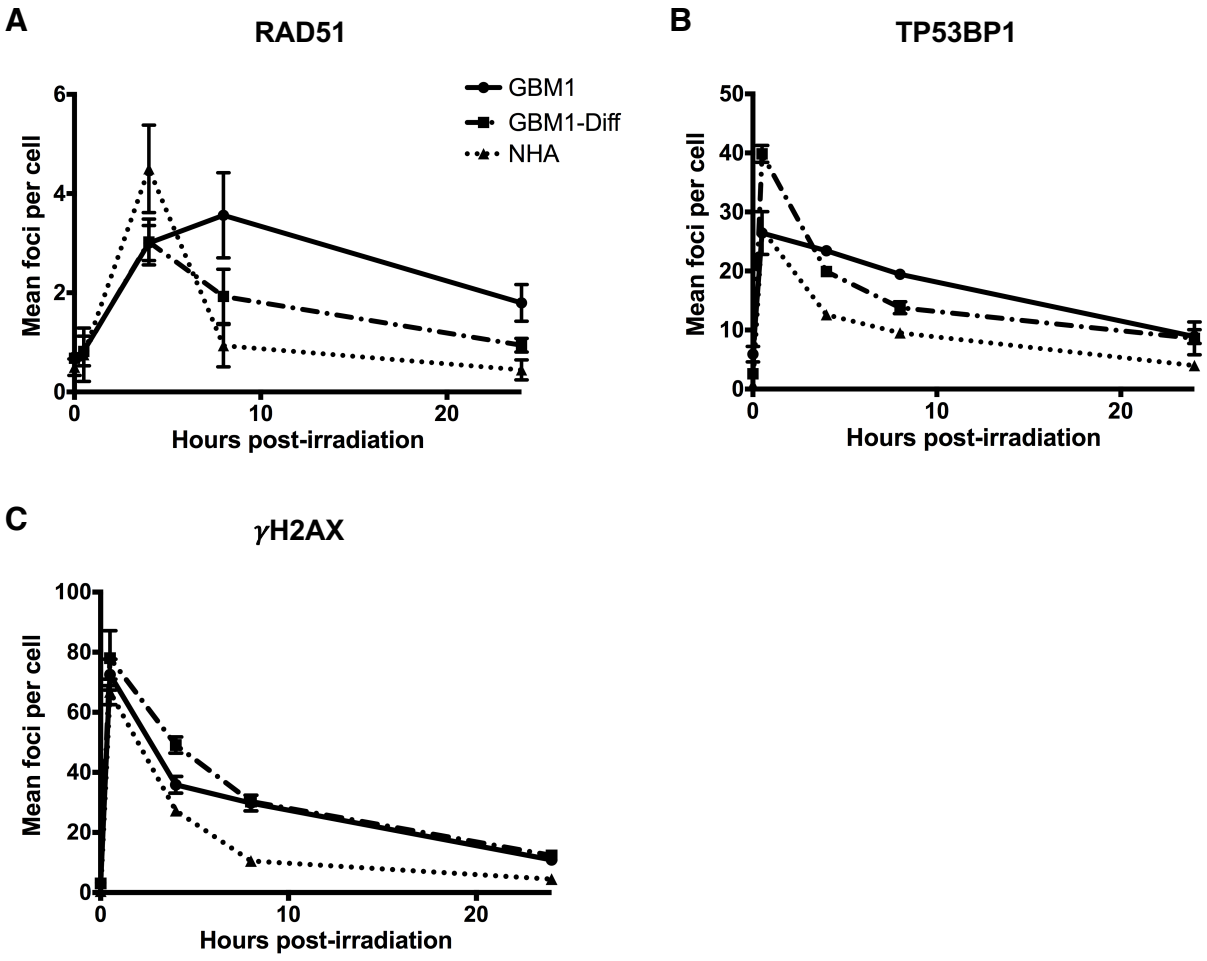


Figure S3, related to Figure 3. Cells were irradiated with 3 Gy and fixed at 30 minutes, 4 hour, 8 hours and 24 hours after treatment. Non-treated cells were fixed at the time of irradiation (0 hour). At each time point, immunofluorescene was used to quantify the number of nuclear foci of **(A)** RAD51, **(B)** TP53BP1 and **(C)** γH2AX proteins. Foci were counted in 50 to 150 cells for each protein at each time point. Error bars = SEM.

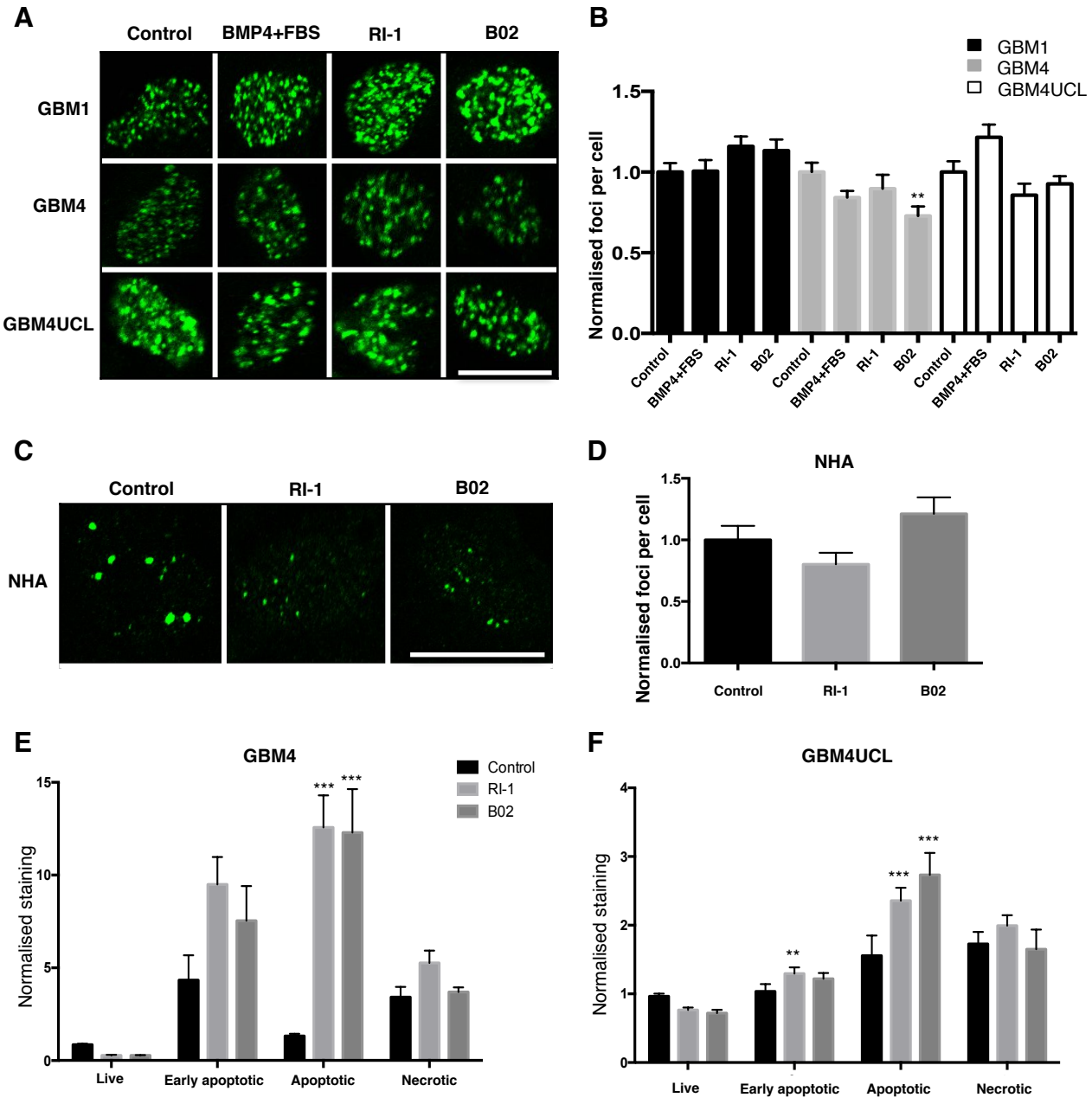
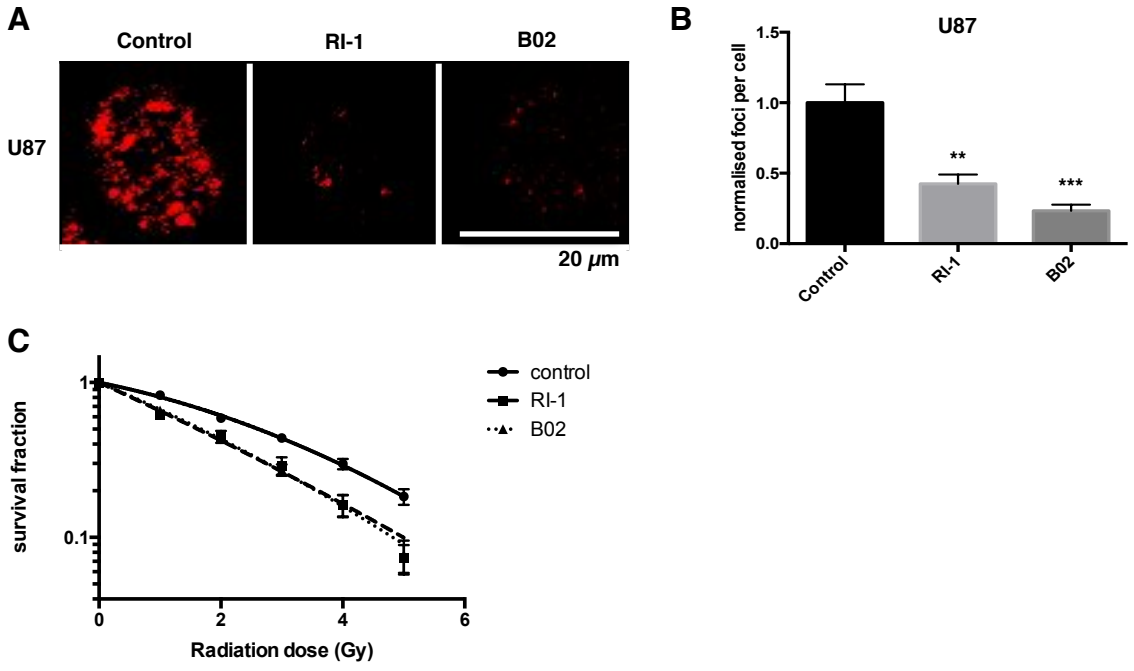


Figure S4, related to Figure 4. (A) GSC lines treated with RAD51 inhibitors were irradiated with 3 Gy. After 30 min the cells were fixed and immunofluorescence (IF) microscopy was used to visualise γH2AX foci (representative images shown). **(B)** Quantification of γH2AX foci from **(A)** (n=3 independent experiments, ≥100 cells counted per treatment). **(C)** IF microscopy was used to visualise γH2AX foci in NHA treated with RI-1, B02 or DMSO, 24 h after irradiation (3 Gy). **(D)** Relative number of foci 24 h after irradiation of NHA treated with RAD51 inhibitors (n=3 independent experiments, ≥100 cells counted per treatment). **(E, F)** Annexin V/PI staining of GBM4 **(E)** or GBM4UCL **(F)** cells treated with B02 or RI-1 (7.5 μM) 24 h prior to irradiation (2 Gy) followed by incubation at 37°C for 5 days prior to staining (n=3 independent experiments). Scale bar = 20 μm. Error bars = SEM. Statistical significance was calculated using one-way ANOVA with ** representing p=<0.05 and *** representing p=<0.001.



MID	Dose modifying factor	α constant
Con- 2.75		Con_{α} 0.184
RI-1- 2.05	RI-1- 1.34	$RI\text{-}1_{\alpha}$ 0.412
B02- 2.07	B02- 1.33	$B02_{\alpha}$ 0.379

Figure S5, related to Figure 4. (A) U87 cells were treated with RI-1 and B02 (30 μM) and then irradiated with 3 Gy. The cells were allowed to recover for 4 h after which RAD51 foci were visualised by immunofluorescence microscopy (representative images shown). **(B)** Quantification of RAD51 foci from **(A)** (n=3 independent experiments with ≥100 cells counted per treatment). **(C)** U87 cells treated with RAD51 inhibitors (RI-1, 1.5 μM or B02, 1.2 μM) were used in a clonogenic survival assay with radiation doses of between 0 and 5 Gy. The data was analysed using a linear-quadratic model of radiation survival giving the mean inactivation dose (MID), dose modifying factor and α (linear) constant (n=3 independent experiments). Error bars = SEM. Statistical significance was calculated using one-way ANOVA with ** representing p=<0.05 and *** representing p=<0.001.