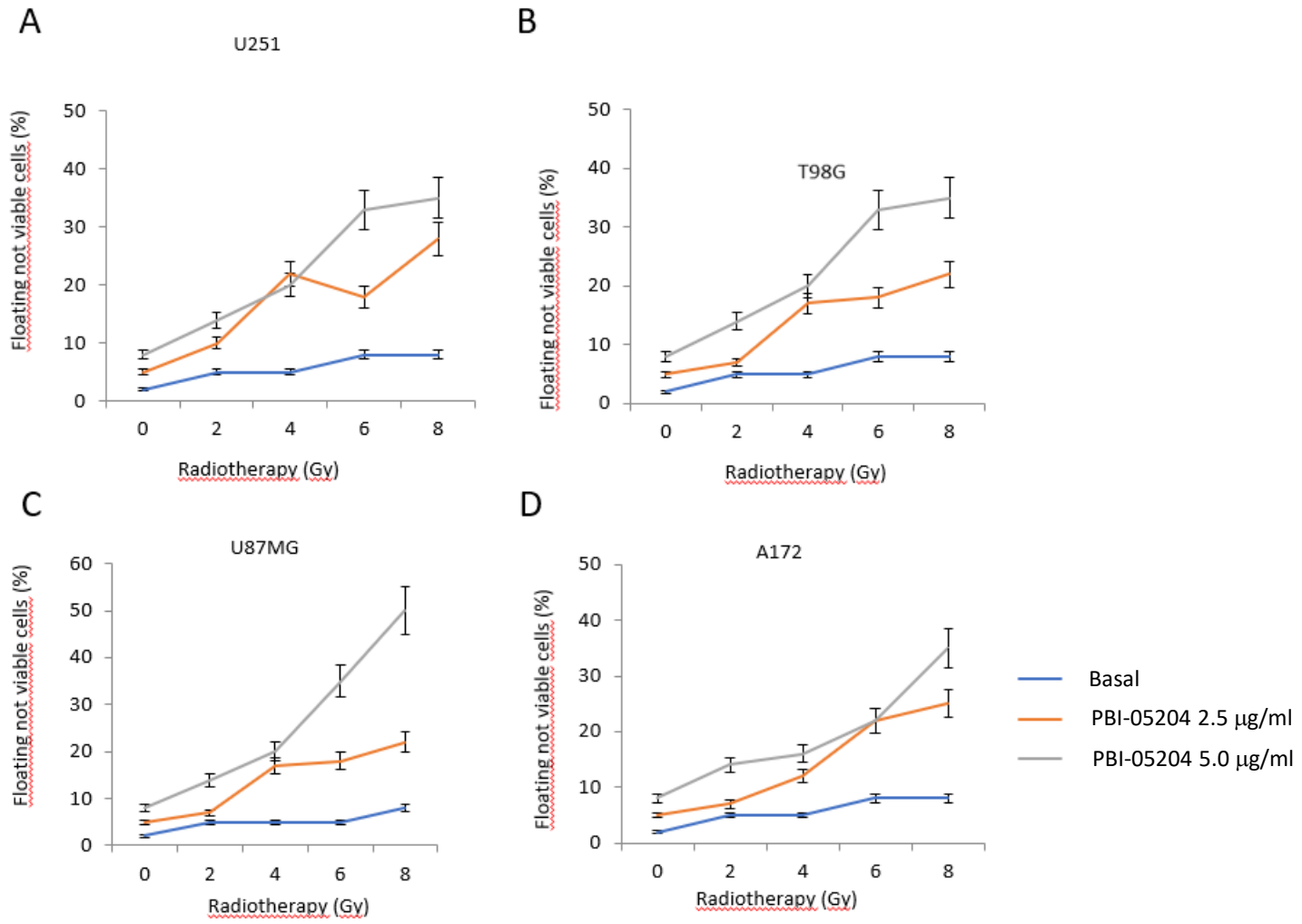



Figure S1. Increased number of non-viable floating cells following treatment with PBI-05204 plus RT in GBM cells.

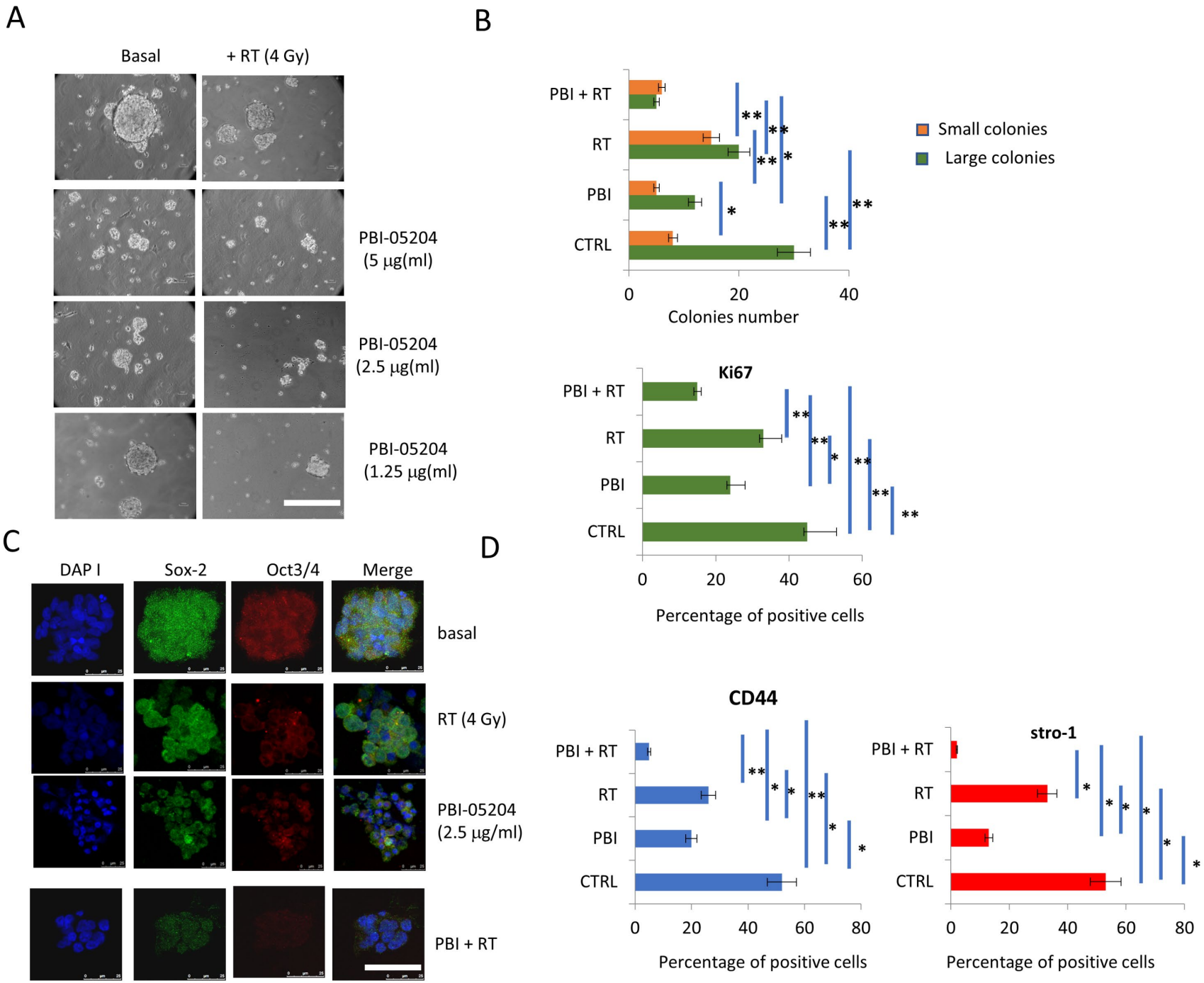

A
Basal
+ RT (4 Gy)
PBI-05204
(5 μg(ml)
PBI-05204
(2.5 μg(ml)
PBI-05204
(1.25 μg(ml)
B
PBI + RT
RT
PBI
CTRL
Small colonies
Large colonies
0
20
40
Colonies number
Ki67
Percentage of positive cells
C
DAP I
Sox-2
Oct3/4
Merge
basal
RT (4 Gy)
PBI-05204
(2.5 μg/ml)
PBI + RT
D
CD44
stro-1
Percentage of positive cells

Figure S2

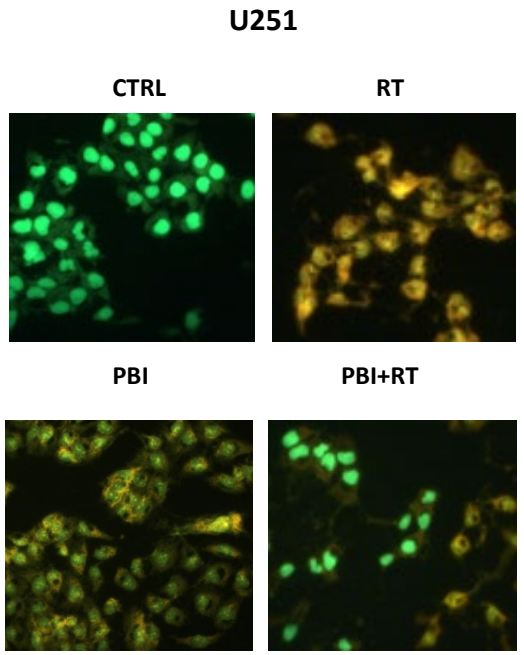
Figure S2. PBI-05204 enhanced RT mediated suppression of cell proliferation and stemness in U87MG cells. U87MG spheroids were cultured for 3 weeks after administration of 1.25, 2.5 or 5.0 µg/ml PBI-05204 alone or after a single dose of RT (4 Gy) administered two days after PBI-05204 treatment. (A) Morphologic appearance of U87MG cells grown as neurosphere. Bar (1 cm) correspond to 500 µm. (B) Count of small (< 20 cells) and large (>20 cells) colonies at 5X magnification (Top). FACS analyses of Ki67 expression in acutase™ treated single cell suspensions (Bottom, n = 3). Data are represented as mean ± SD. *$p<0.05$, ** $p<0.01$. (C) Confocal analysis of expression of stem cells markers SOX2 and Oct3/4 after the cells were treated with control, RT only, PBI-05204 (2.5 µg/ml) and combination of RT and PBI-05204 for 96 hrs. Bar represents 25 µm. (D) The FACS analysis of stro1 and CD44 levels in U87MG cells. Data are presented as mean ± SE of three separate experiments. *$p<0.05$, ** $p<0.01$.

Table S1: Summary of comet assay results analyzed by CaspLab software

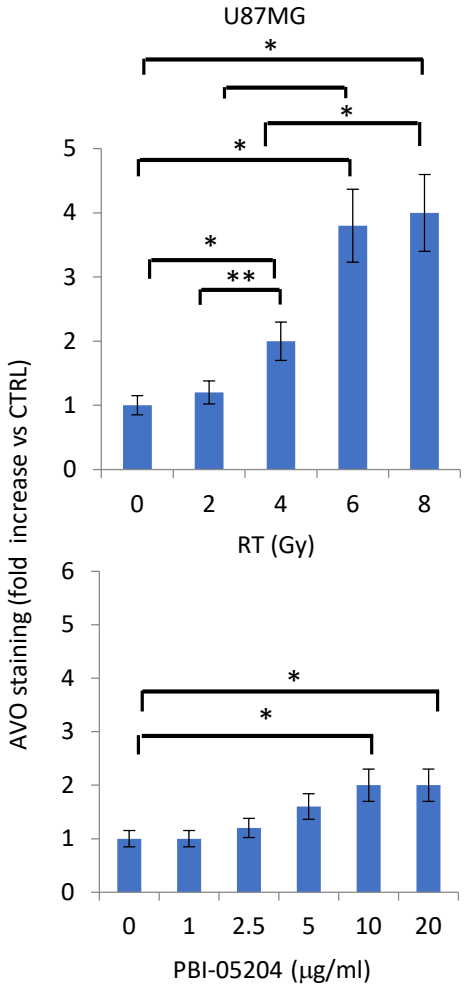
| U87MG | | | |
|---|---|---|---|
| Condition | DNA Damage (% DNA in tails) | Tail length (μm) | Total DNA damage (tail moment arbitrary units) |
| CTRL | 4.0 ± 0.5 | 6.8 ± 0.5 | 2.5 ± 0.2 |
| RT 1 hr | 15.5 ± 5.8* | 20.0 ± 2.0* | 12.8 ± 4.2* |
| PBI-05204 1 hr | 5.3 ±3.8 | 15.3 ± 1.5* | 5.8 ± 3.0 |
| RT + PBI 1hr | 35.5 ± 5.5* | 45.5 ± 2.3* | 35.4 ± 7.8* |
| RT 18 hr | 28.0 ± 6.0* | 53.5 ± 5.5*,a | 38.5 ± 4.2*, a |
| RT + PBI 18 hr | 48.0 ± 6.5*, b, ** | 73.5 ± 10.3*, b.** | 55.6 ± 5.8 * |
| **U251** | | | |
| CTRL | 4.0 ± 0.2 | 3.0 ± 0.5 | 2.5 ± 0.4 |
| RT 1 hr | 9.7 ± 3.0 | 17.8 ± 2.8* | 8.9 ± 2.7* |
| PBI-05204 1 hr | 12.7 ± 0.8* | 22.5 ± 6* | 5.0 ± 3.5 |
| RT + PBI 1hr | 29.0 ± 6.3* | 40.9 ± 3.5* | 29.7 ± 8*,α |
| RT 18 hr | 19.7.0 ± 5.3* | 37.0 ± 8.5* | 33.57± 5.6* |
| RT + PBI 18 hr | 52.0 ± 8.7* | 58.6 ± 8.6* | 60.3 ± 6.4*,b, c |

*p<0.01 vs CTRL, [a]p<0.01 vs PBI or RT alone; [b] p<0.01 vs RT 18 hr, [c] p<0.01 vs RT + PBI 1 hr

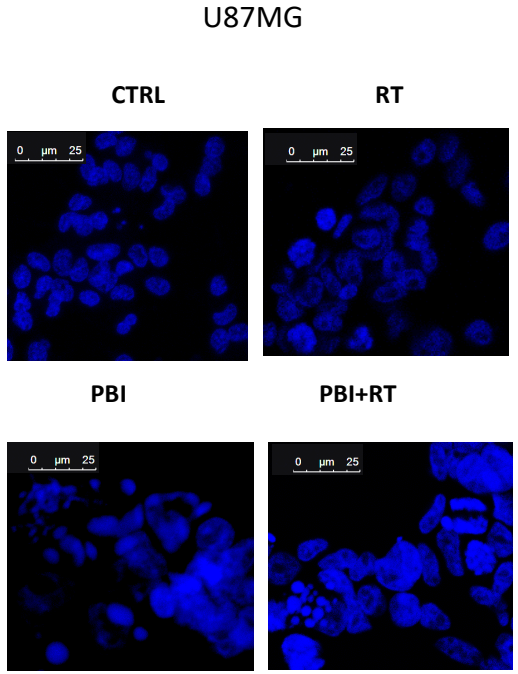
A


U251
CTRL
RT
PBI
PBI+RT

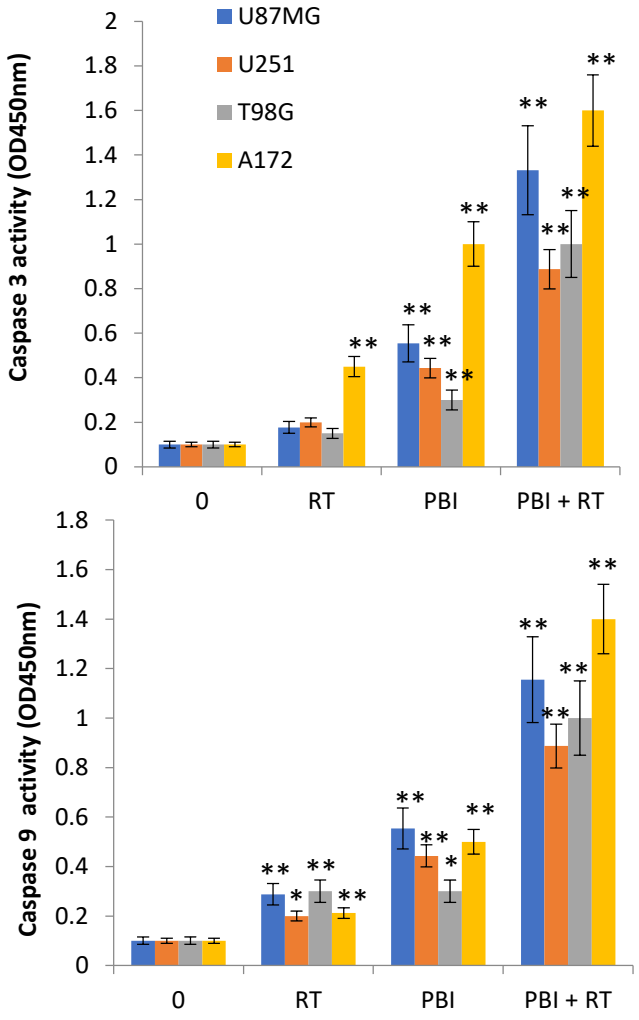
B


U87MG
AVO staining (fold increase vs CTRL)
RT (Gy)
0
2
4
6
8
PBI-05204 (µg/ml)
0
1
2.5
5
10
20

C

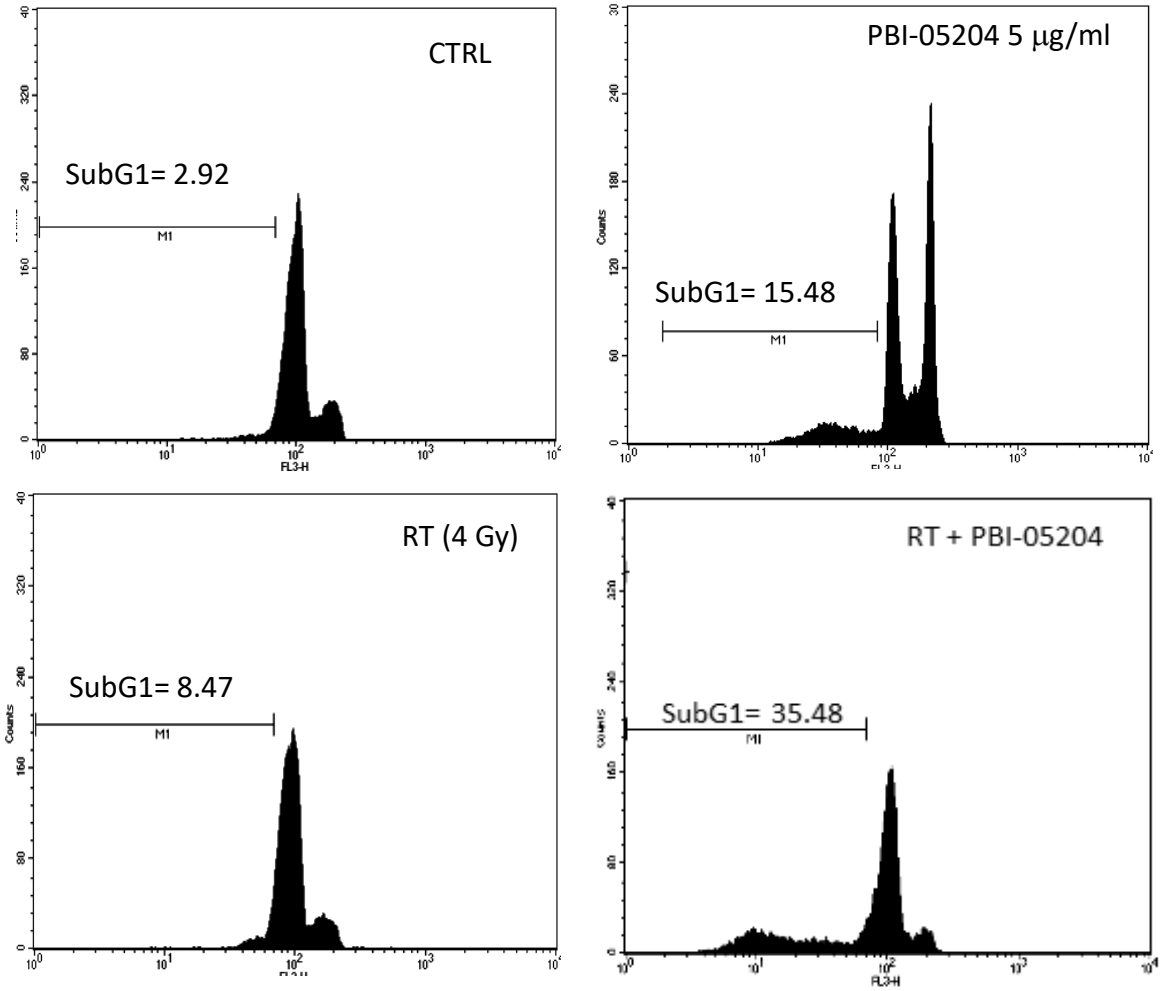

U87MG
CTRL
RT
PBI
PBI+RT

D


U87MG
U251
T98G
A172
Caspase 3 activity (OD450nm)
Caspase 9 activity (OD450nm)
0
RT
PBI
PBI + RT

E

U87MG


CTRL
SubG1= 2.92
M1
PBI-05204 5 µg/ml
SubG1= 15.48
M1
RT (4 Gy)
SubG1= 8.47
M1
RT + PBI-05204
SubG1= 35.48
M1
FL3-H
Counts

T98G

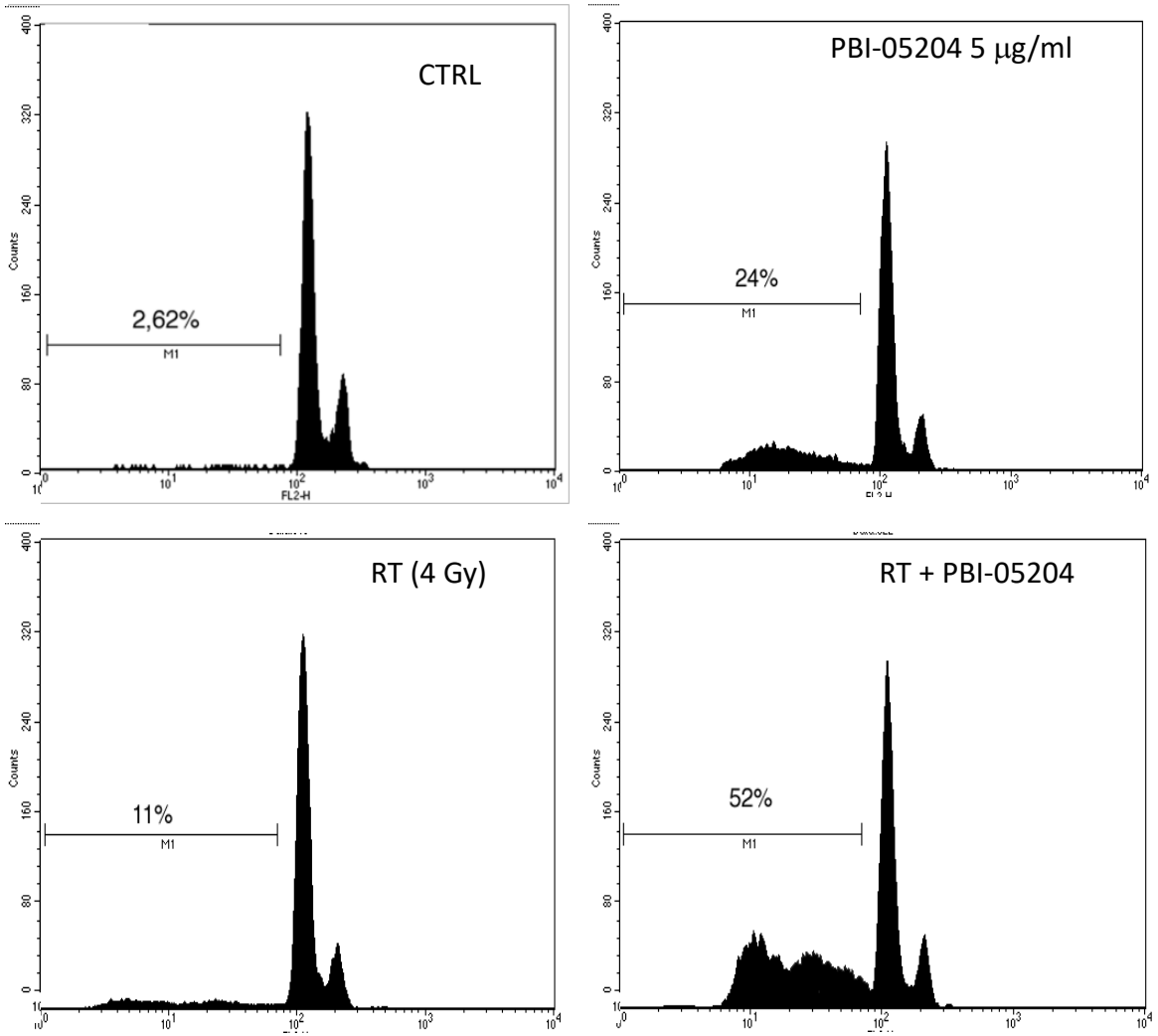

CTRL
2,62%
M1
PBI-05204 5 µg/ml
24%
M1
RT (4 Gy)
11%
M1
RT + PBI-05204
52%
M1
FL2-H
Counts

**Figure S3.** Combination of PBI-05204 and radiotherapy leads to apoptotic cell death in GBM cells.
(A) Representative image of AVO staining performed in U251 cell line treated with RT, PBI-05204 and RT plus PBI-05204. (B) Quantitation of Acridine Orange Staining (AVO) performed in U87MG cells treated with different doses of RT (0-6 Gy) and PBI-05204 (1-20 μg/ml). AVO accumulation was measured by the fold increase/decrease vs CTRL and data are presented as mean ± SE from three experiments. * $p < 0.01$, **$p<0.05$. (C) Apoptotic cell death in U251 cells was confirmed by Hoechst 3324 staining (n = 3 separate experiments). (D) Enzymatic caspase 3 and caspase 8 activation in GBM cells after treatment with RT, PBI-05204 and RT + PBI-05204 for 24 hr ( n = 3 separate experiments). Data are shown as mean ± SE. **$p< 0.01$.
(E) Cell cycle analysis of U87MG and T98G cells treated with RT, PBI-05204 and RT plus PBI-05204 for 24 hrs.

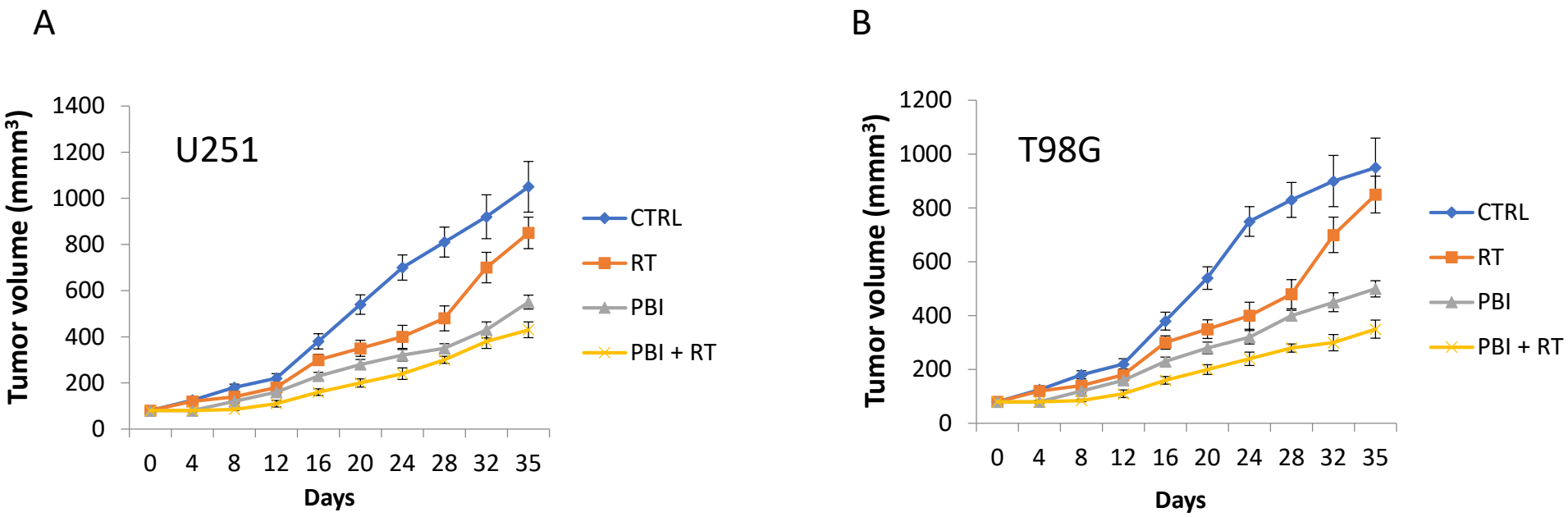



Figure S4. The tumor growth curve of U251 (A) and T98G (B) mouse xenografts after being treated with RT alone, PBI-05204 alone or RT plus PBI-05204. The detailed treatment schedule was described in M&M section. Data are presented as mean ± SD.