hours later by 6 Gy radiation), or DMSO control. Annexin V expression was measured 72 hours after the last treatment.

**Figure 4**: PLX4720 augments radiation antitumor effects *in vivo.* (A) BRAF V600E, firefly luciferase modified, intracranial xenograft model (AM-38) mice were randomized to control (DMSO); PLX4720 alone (10 mg/kg over 14 consecutive days); radiation alone (XRT 1 Gy x 3 on alternating days); concurrent PLX4720 (10 mg/kg over 14 consecutive days) + radiation (1 Gy x 3 on alternating days); or sequential XRT (1 Gy x 3 on alternating days), followed by PLX4720 (10 mg/kg over 14 consecutive days). Combined PLX4720 and radiation therapy produced statistically significant prolonged survival compared to respective monotherapies. P values: Concurrent treatment vs control $p<0.0001$; vs PLX4720 $p=0.0170$; vs radiation $p=0.0077$. Sequential treatment vs control $p<0.0001$; vs PLX4720 $p=0.0158$; vs radiation $p=0.0059$. There was no difference in survival between concurrent and sequential arms ($p=0.57$**).** **(B and C)** For immunohistochemical analyses, BRAF V600E intracranial xenograft model (AM-38) mice were sacrificed 6 hours after the final treatment. PLX4720 + radiation significantly reduced tumor proliferation *in vivo*, assessed by Ki-67 staining, compared to control and each monotherapy. Combination therapy significantly decreased pMAPK and increased p21 and γH2AX levels compared to control but not compared to single agents. P values are shown in Supplemental Table 2.

**Supplemental Figure 1.** Radiation cooperates with PLX4720 to reduce S phase and induce apoptosis in BRAF V600E glioma cells. Flow cytometry analyses of (A-B) cell cycle using

BrdU and 7-AAD staining; and (C-D) apoptosis using Annexin V staining. Cells analyzed include (A, C) BRAF WT GBM36 and (B, D) BRAF V600E DBTRG-05MG cells. These additional cell lines complement the data shown in Figures 2 and 3 and utilize identical methods to those delineated in Figures 2 and 3.

**Supplemental Figure 2**. Flow cytometric *in vitro* measurement of β-galactosidase levels in (A) GBM36 (BRAF WT) and (B) AM-38 (BRAF V600E) cells reveals higher background levels of senescence in BRAF V600E cells compared to BRAF WT cells but no pronounced effects of PLX4720, radiation or their combination on levels of senescent cells.

**Supplemental Figure 3.** Immunohistochemical analyses of survival study mice reveals combination (concurrent) therapy of PLX4720 and radiation significantly decreases tumor proliferation (Ki-67 staining) and increases cleaved caspase 3 (CC3) compared to control mice. Comparisons of combination therapy to each monotherapy were not statistically significant.

**Supplemental Table 1.** BRAF V600E inhibition by PLX4720 leads to growth inhibition in BRAF V600E cell lines, but IC50 values were not reached in BRAF WT glioma lines.

| Cell Line | Genotype | IC50 of PLX4720 by cell viability | IC50 of PLX4720 by clonogenics |
|---|---|---|---|
| **DBTRG-05MG** | V600E | 2 uM | 2 uM |
| **AM-38** | V600E | 1 uM | 0.5 uM |
| **GBM8** | WT | Not reached at 20 uM – increased viability | Growth activation |
| **GBM39** | WT | Not reached at 20 uM – increased viability | Growth activation |

**Supplemental Table 2.** T-test comparisons of Ki67, pMAPK, p21 and γH2AX immunohistochemical staining of BRAF V600E tumor xenograft sections treated with PLX4720, radiation or combination (sequential) therapy assessed 6 hours after completion of treatment.

| T-test comparisons p-values | Control vs. PLX4720 | Control vs. XRT | Control vs. Combination | PLX4720 vs. XRT | PLX4720 vs. Combination | XRT vs. Combination |
|---|---|---|---|---|---|---|
| **Ki67** | 0.61 | 1.0 | **0.00034** | 1.0 | **0.018** | **0.010** |
| **pMAPK** | 0.084 | **0.00073** | **0.00017** | 0.31 | 0.064 | 1.0 |
| **p21** | 0.27 | **0.050** | **0.0012** | 1.0 | 0.14 | 0.72 |
| **γH2AX** | 0.20 | 0.095 | **0.022** | 1.0 | 1.0 | 1.0 |

Supplementary Figure 1

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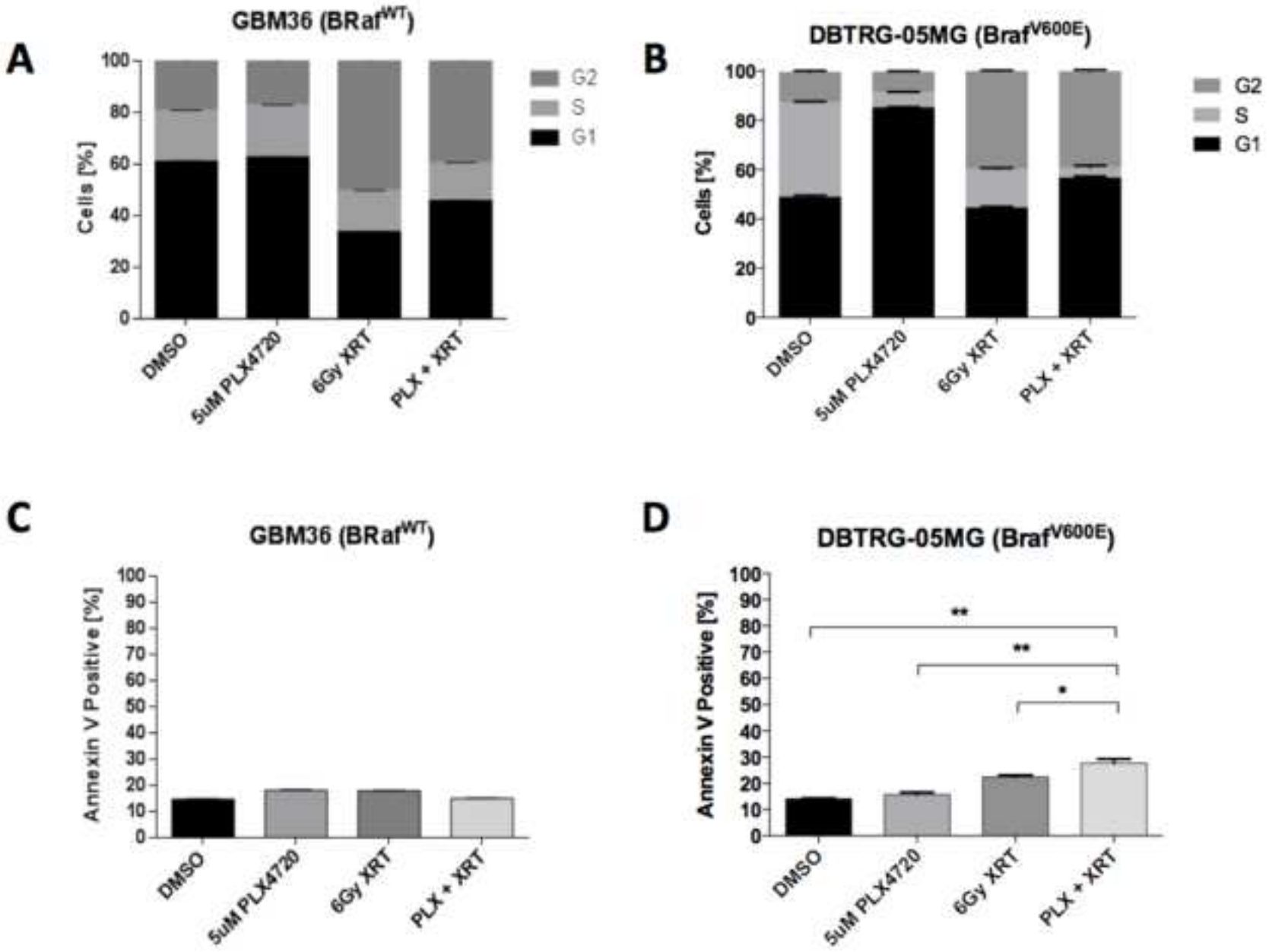
# Supplemental Figure 1



Supplementary Figure 2

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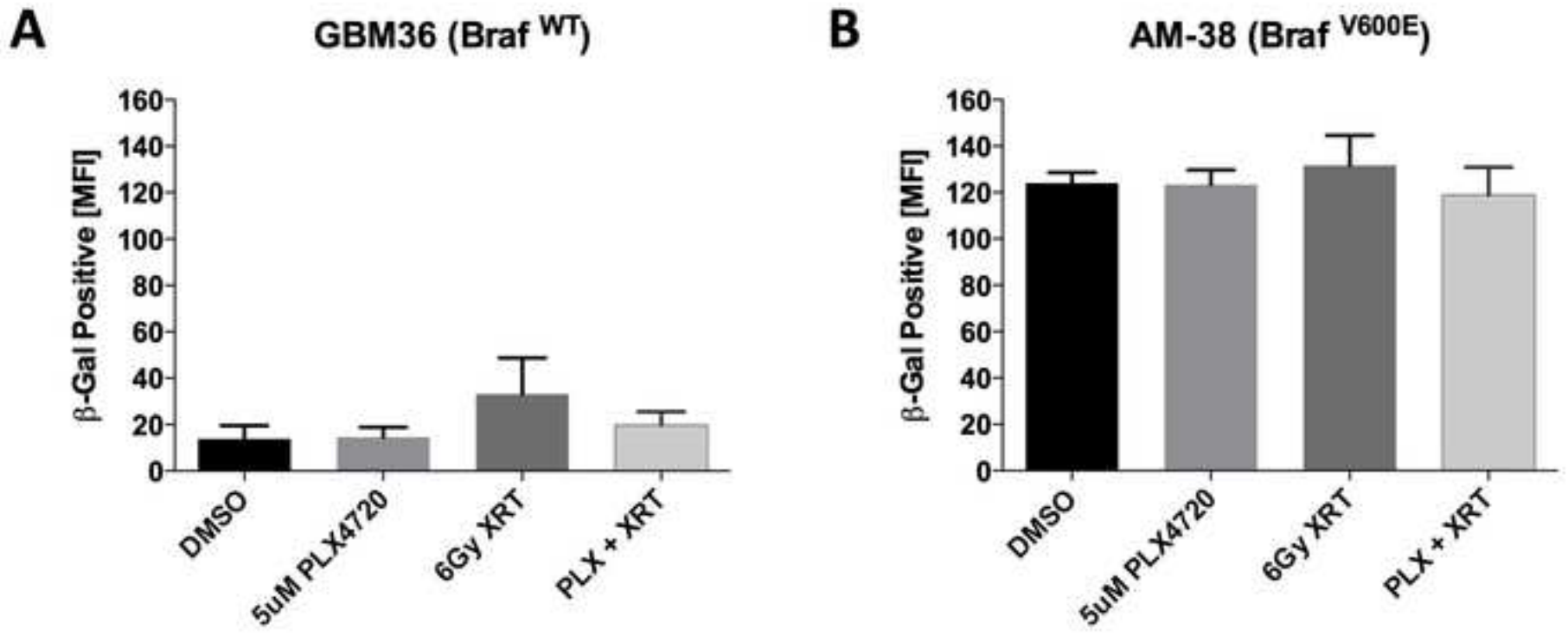
# Supplemental Figure 2



Supplementary Figure 3

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## Supplemental Figure 3