Supplementary Tables

Supplementary Table 1. Inhibitors of DNA damage response pathways used in these studies*

Chemical Compound	Inhibitory Effect on	Concentration Used in the Study
NU7021	DNA-PKcs	2.5 µM
KU55933	ATM kinase	~20 µM
CGK733	ATM / ATR kinase	~5 µM
lithocholic acid (LCA)	PARP	50 µM
PARP inhibitor XIV	PARP	2 µM
UCN-01	Chk1	~50 nM
ChkII inhibitor II	Chk2	1 µM
triciribine	Akt	5 µM
3-methyladenine (3-MA)	autophagy	1 mM

*DNA-PKcs = DNA-dependent protein kinase, catalytic subunit; ATM = ataxia telangiectasia mutated; ATR = ataxia telangiectasia and Rad3-related; PARP = poly(ADP-ribose) polymerase; Chk1 = checkpoint kinase 1; Chk2 = checkpoint kinase 2.

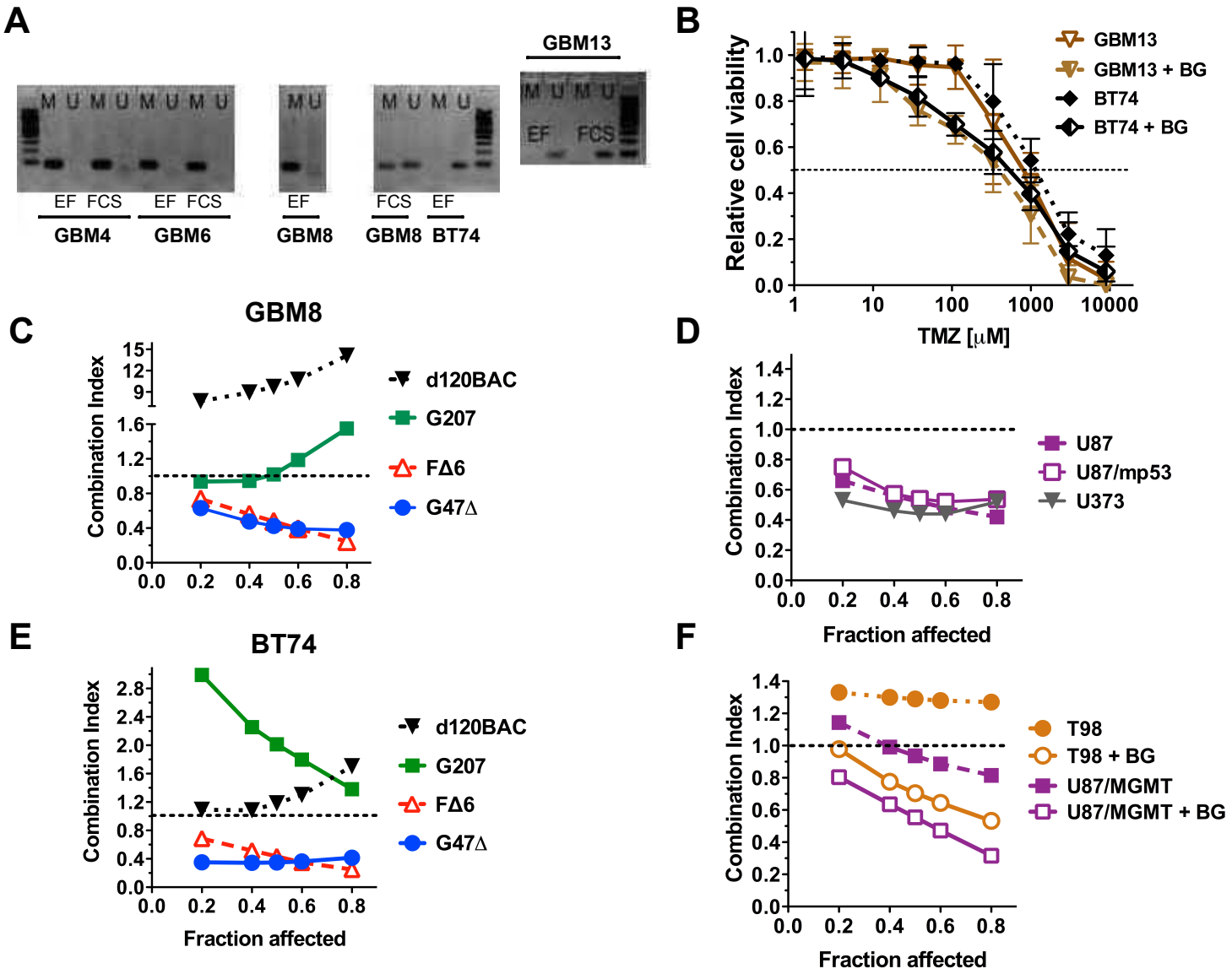
Supplementary Table 2. Effect of DNA damage response inhibition on median-effect doses of TMZ and G47Δ in vitro*

Cell line	ED_{50}: TMZ or G47Δ	
	TMZ, μM	G47Δ, MOI
U87		
without inhibitor	300	0.08
+ NU7026	268	0.07
+ KU55933	202	0.14
+ CGK733	153	0.14
+ LCA	266	0.08
+ PARP inhibitor XIV	274	0.08
+ UCN-01	197	0.08
+ Chk2 inhibitor II	268	0.08
+ triciribine	275	0.08
+ 3-MA	377	0.1
U373		
without inhibitor	230	0.08
+ NU7026	200	0.08
+ KU55933	152	0.13
+ CGK733	141	0.14
GBM4		
without inhibitor	32	0.21
+ KU55933	13	0.31
+ CGK733	10	0.3
shRNA		
Non-target	41	0.2
ATM	21	0.29
ATR	30	0.2
ATM/ATR	13	0.3
MSH6	408	0.25
GBM8		
without inhibitor	5.8	0.1
+ KU55933	1.9	0.19
+ CGK733	1.3	0.21
shRNA		
Non-target	5.2	0.1
ATM	2.2	0.16
ATR	2.8	0.1
ATM/ATR	1.9	0.15
GBM13 + BG		
without inhibitor	325	0.05
+ KU55933	192	0.07
+ CGK733	111	0.09

BT74 + BG		
without inhibitor	415	0.23
+ KU55933	222	0.31
+ CGK733	194	0.31
shRNA		
Non-target	450	0.24
ATM	220	0.31
ATR	425	0.23
ATM/ATR	201	0.29

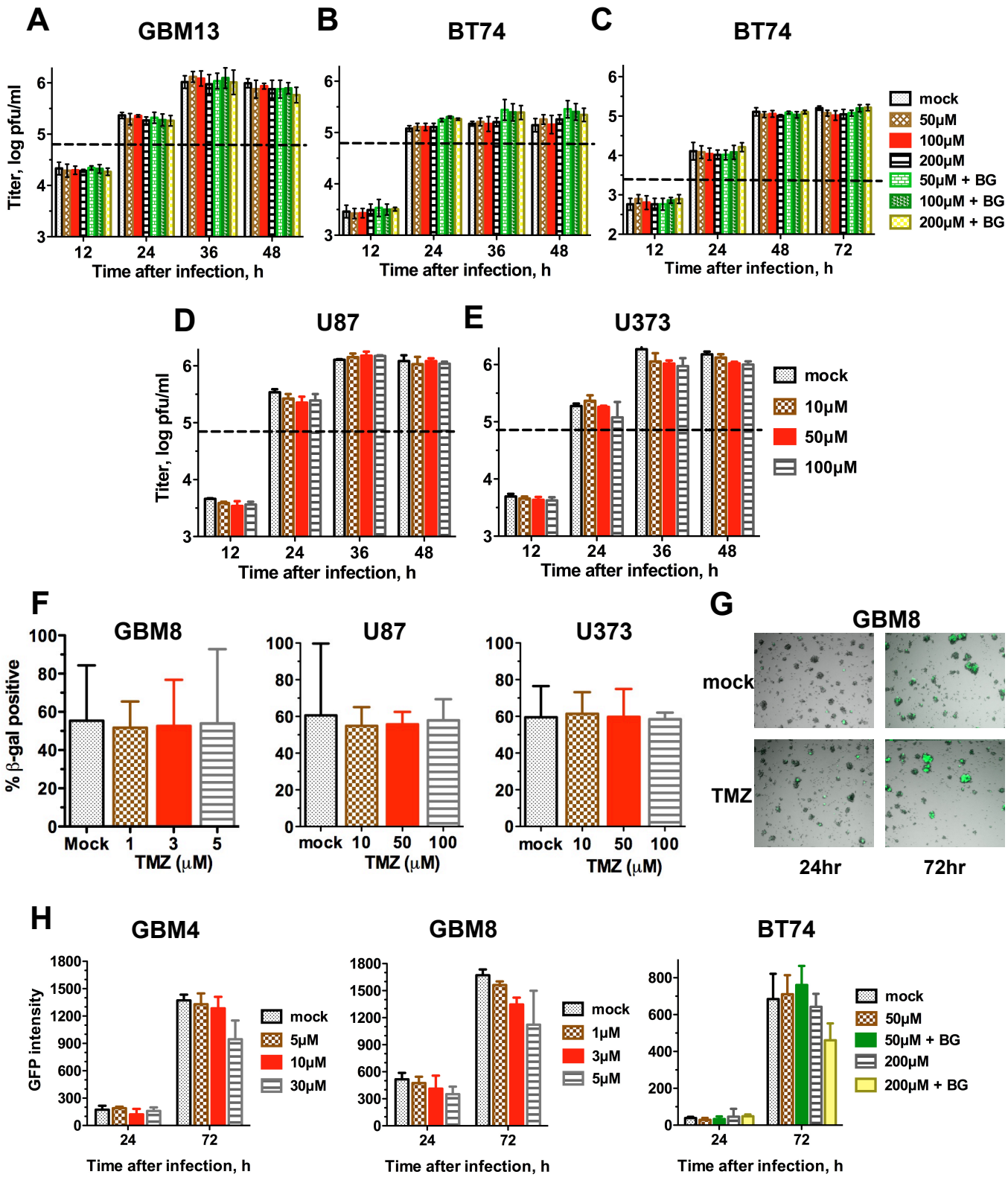
*Inhibitory doses (ED_{50}) for TMZ and G47Δ in the presence of pharmacological inhibitors (non-toxic concentrations from Supplementary Table 1) or shRNA in GSCs and glioma cell lines. Sensitivity to TMZ was determined 5 days after adding TMZ. Sensitivity to G47Δ was determined 4.5 days after infection. ED_{50} (dose required for 50% effect) values were determined from dose–response curves. TMZ = temozolomide; BG = O^6-benzylguanine; DNA-PKcs = DNA-dependent protein kinase, catalytic subunit; PARP = poly(ADP-ribose) polymerase;ATM = ataxia telangiectasia mutated; ATR = ataxia telangiectasia and Rad3-related.

Supplementary Figure 1



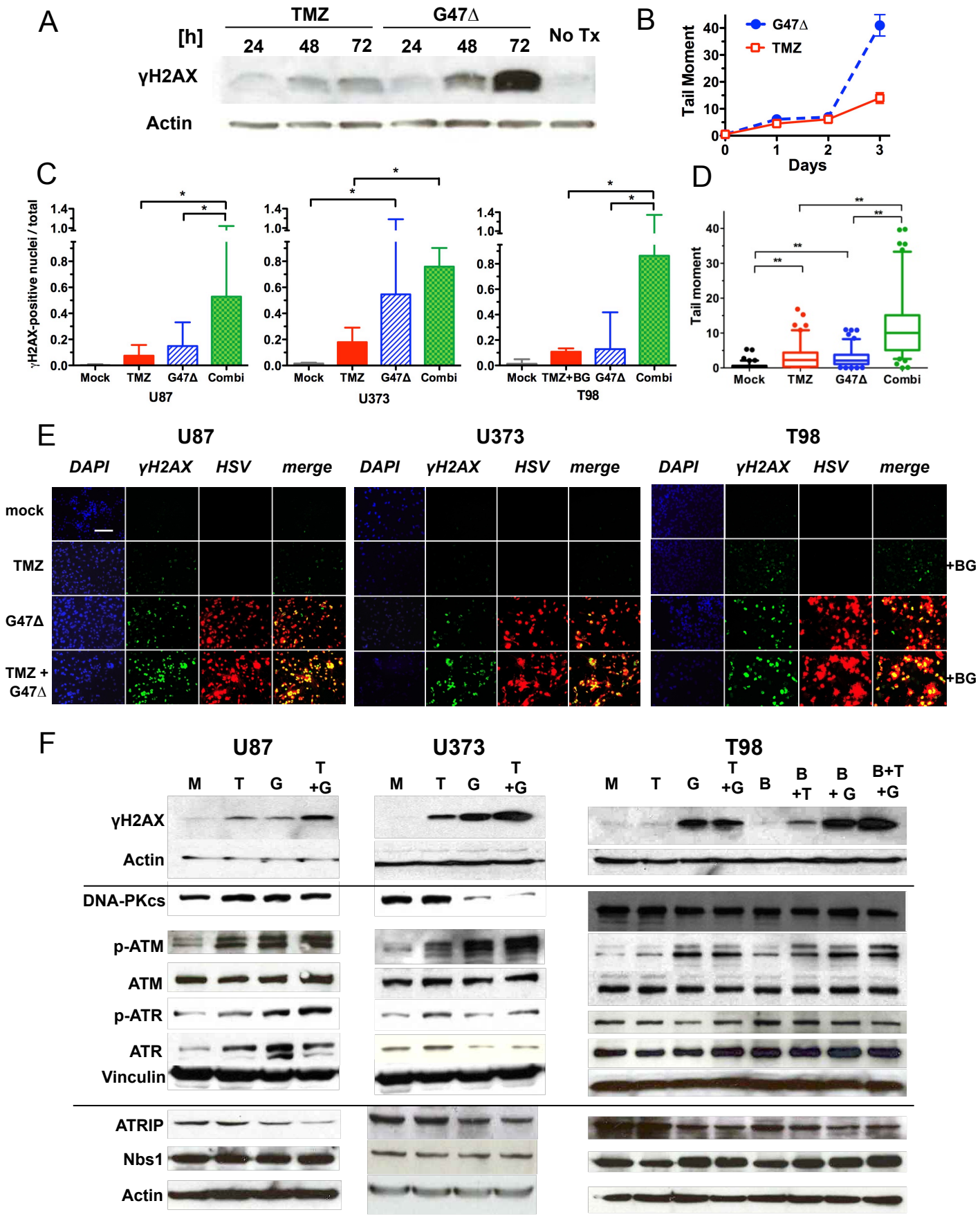
Supplementary Figure 1. MGMT status of GSCs and interaction of TMZ and HSV in glioma cell killing. **A**) DNA methylation status of MGMT promoter was examined by methylation specific polymerase chain reaction (MSP). EF and FCS denote GSCs and GBM primary cells cultured in serum-containing media, respectively, from the same patient specimen. M and U denote methylated and unmethylated, respectively. **B)** Dose response curves for TMZ in MGMT-positive GSCs and with BG (50μM, 2 h before TMZ). Cell viability was measured by MTS assay. Error bars represent 95% confidence intervals. **C-F**) Interaction of TMZ and HSV in GSC and glioma cell line killing examined by the median effect method of Chou-Talalay. Data are shown as Fraction affected–Combination Index (CI) plots. CI < 1, CI = 1, and CI > 1 represent synergistic, additive, and antagonistic interactions respectively. **C**) Comparison between G47Δ and HSV mutants G207, FΔ6, and d120BAC in GBM8. **D)** The interaction of TMZ and G47Δ in MGMT-negative glioma cell lines, including U87 expressing mutant p53. **E)** Comparison between G47Δ and HSV mutants G207, FΔ6, and d120BAC in BT74 in the presence of BG (50μM). **F**) The interaction of TMZ and G47Δ in MGMT-positive glioma cell lines, and with BG (20μM). GSC = glioblastoma stem cell; TMZ = temozolomide; MGMT = O^6-methylguanine-DNA-methyltransferase; BG = O^6benzylguanine.

Supplementary Figure 2



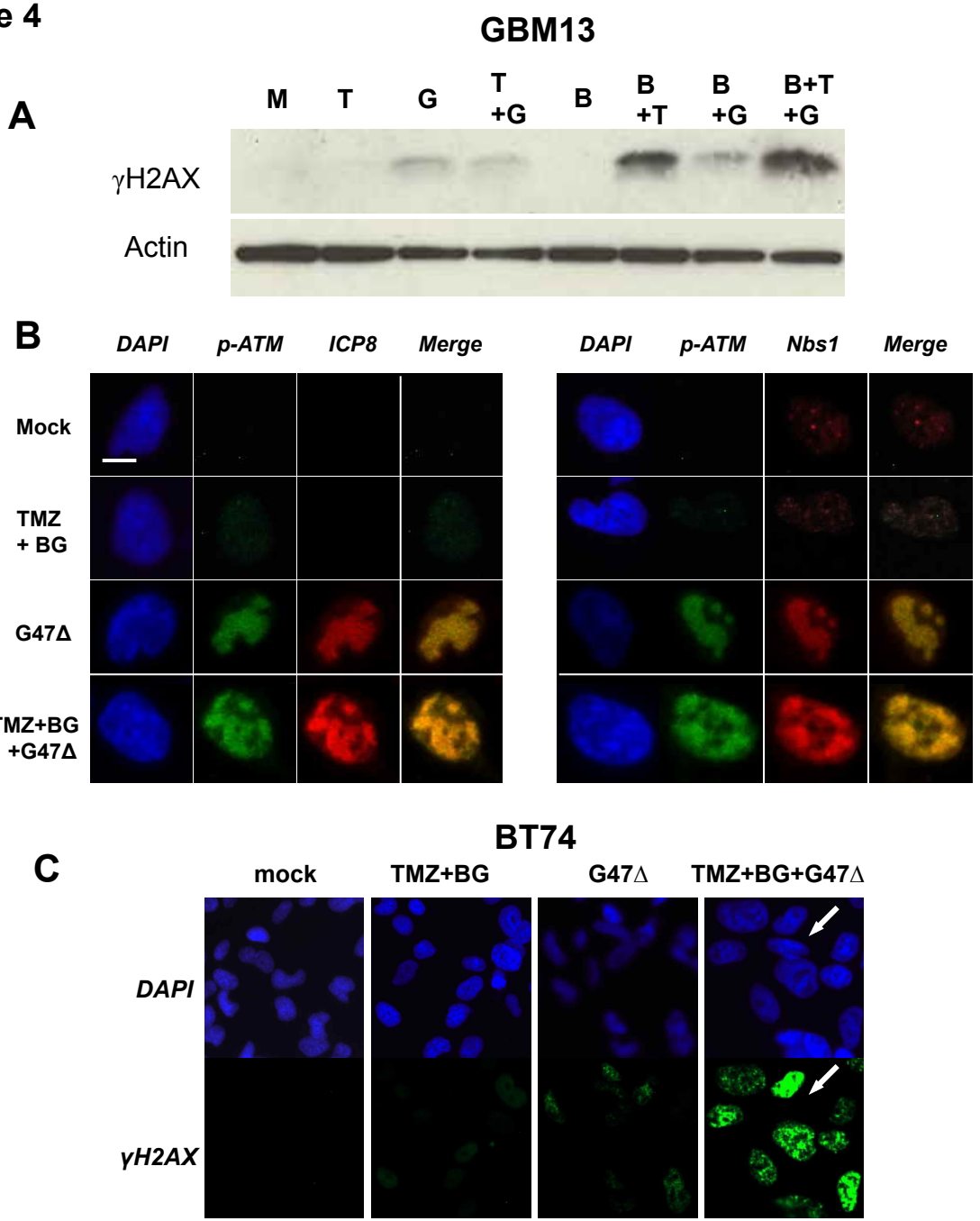
Supplementary Figure 2. Effect of TMZ on G47Δ replication, infectivity and spread in GBM cells in vitro. **A-E**) G47Δ replication was examined in the presence of indicated concentrations of TMZ. Cells (GBM13, **A**; BT74, **B, C**; U87, **D**; U373, **E**) were treated with TMZ 24 hours before infection with G47Δ at MOI=1.5 (**A, B, D, E**) or MOI=0.1 (**C**). MGMT-positive GBM13 and BT74 were also treated with indicated concentrations of TMZ and BG (50μM). At the indicated times after infection, cells and media were collected and virus titers were determined by plaque assay on Vero cells. Error bars represent 95% confidence intervals. Dashed lines indicate the dose of G47Δ used for infection. There were no statistically significant differences between mock and high dose TMZ (unpaired t-test, two-sided). **F)** G47Δ infectivity was examined in the presence of indicated TMZ concentrations. The proportion of X-gal staining cells was determined (% β-galactosidase positive). Error bars represent 95% confidence intervals. **Left:** GBM8, **Middle:** U87, **Right:** U373. There were no statistically significant differences between mock and high dose TMZ (unpaired t-test, two-sided). **G, H)** GSCs were infected with EGFP-expressing G47ΔBAC at MOI=0.1 in the presence of indicated concentrations of TMZ or TMZ+BG (50μM). **G)** Representative microscope images (phase contrast overlaid with fluorescence, green for EGFP) for GBM8. **H)** The GFP signal intensity was measured as an indication of viral spread. Error bars represent 95% confidence intervals. **Left:** GBM4, **Middle:** GBM8, **Right:** BT74. At 72 hours, high TMZ doses killed cells and thus decreased GFP intensity. GBM = glioblastoma; TMZ = temozolomide; MOI = multiplicity of infection; BG = O^6-benzylguanine; MGMT = O^6-methylguanine-DNA-methyltransferase; GSCs = glioblastoma stem cells; EGFP = enhanced green fluorescent protein.

Supplementary Figure 3



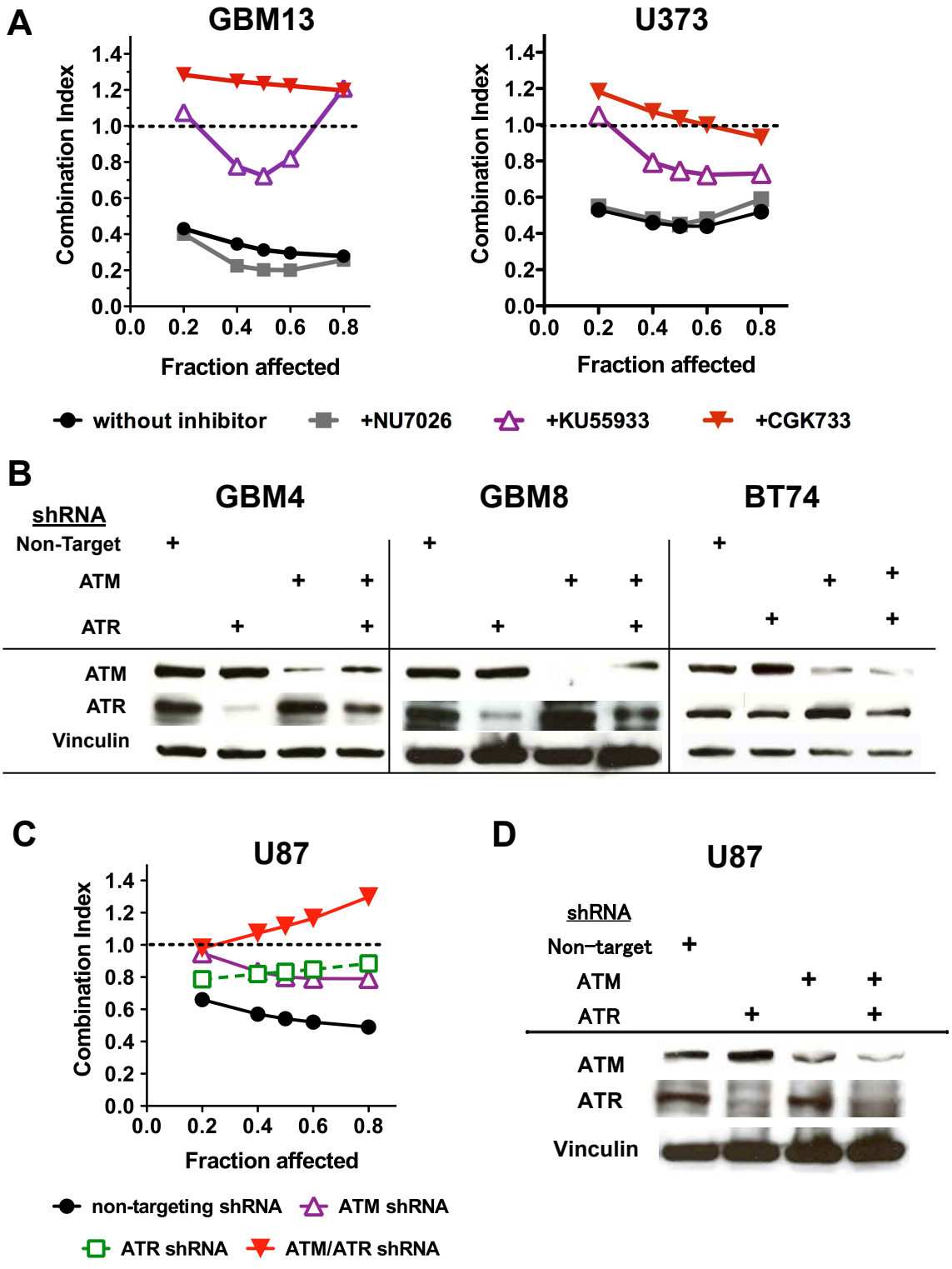
Supplementary Figure 3. Induction of DNA damage and DNA damage responses in glioma cell lines in vitro. **A)** Induction of γH2AX by TMZ or by G47Δ. U87 cells were treated with TMZ (50μM) or G47Δ (MOI=0.1), collected at the indicated times after treatment, and processed for western blotting. **B**) U87 cells were treated with TMZ (50 μM) or G47Δ (MOI=0.1). Each day after treatment, cells were collected and processed for the neutral comet assay. Error bars represent 95% confidence intervals. **C**) γH2AX positive cells/total cells in U87 (**Left**), U373 (**Middle)**, and T98 (**Right**) were quantified by counting in three randomly selected fields. Error bars represent 95% confidence intervals. Asterisks denote statistically significant differences (1 way ANOVA, Bonferroni's Multiple Comparison Test, $P < .05$). For U87, Mock vs TMZ: difference = -0.073, 95% CI = -0.13 to -0.02, $P = .02$; Mock vs G47Δ: difference = -0.15, 95% CI= -0.26 to -0.03, $P = 0.02$; TMZ vs Combi: difference = -0.45, 95% CI = -0.79 to -0.12, $P = .02$; G47Δ vs Combi: difference = -0.38, 95% CI = -0.73 to -0.027, $P = .04$. For U373, Mock vs TMZ: difference = -.16, 95% CI = -0.24 to 0.092, $P = .003$; Mock vs G47Δ: difference = -0.53, 95% CI = -0.94 to -0.12, $P = .02$; TMZ vs Combi: difference = -0.58, 95% CI = -0.70 to -0.46, $P < .001$. For T98, Mock vs TMZ + BG: difference = -0.095, 95% CI = -0.12 to -0.066, $P < .001$; TMZ+BG vs Combi: difference = -0.75, 95% CI = -1.06 to -0.44, $P = .002$; G47Δ vs Combi: difference = -0.73, 95% CI = -1.1 to -0.37, $P = .004$ (unpaired *t*-test, two-sided). **D**) DNA damage was assessed by neutral comet assay. U87 cells were treated with TMZ (50μM) for 36 hours, then infected with G47Δ (MOI=1), and processed for the neutral comet assay 24 h later. Asterisks denote statistically significant differences (1 way ANOVA, Bonferroni's Multiple Comparison Test, $P < .05$). For U87, Mock vs TMZ: difference = -2.6, 95% CI= -3.4 to -1.9, $P < .001$; Mock vs G47Δ: difference= -2.3, 95% CI = -2.9 to -1.8, $P < .001$; TMZ vs Combi: difference = -4.4, 95% CI= -10 to -6.6, $P < .001$; G47Δ vs Combi: difference = -8.7, 95% CI = -10 to 7.0, $P < .001$ (unpaired *t*-test, two-sided). **E**) MGMT-negative U87 (**Left)** and U373 (**Middle**) glioma cells were mock-treated or treated with TMZ (50μM). MGMT-positive T98 (**Right**) glioma cells were mock-treated or treated with TMZ (200μM) + BG (20 μM). 36 hours after TMZ or TMZ + BG, cells were infected with mock or G47Δ at MOI=1, and 24 hours later, cells were fixed and processed for immunocytochemistry (DAPI, blue; γH2AX, green; HSV, red; merge, yellow). Scale bar = 100μm. **F**) Cells were treated as in **E**, except processed for western blotting. M: mock, T = TMZ, G = G47Δ, B = BG. TMZ = temozolomide; BG = O^6-benzylguanine; MGMT = O^6-methylguanine-DNAmethyltransferase; MOI = multiplicity of infection; DAPI = 4',6-diamidino-2-phenylindole; ATM = ataxia telangiectasia mutated; ATR = ataxia telangiectasia and Rad3-related; p-ATM = phosphorylated ATM (Ser1981); p-ATR = phosphorylated ATR (Ser428); ATRIP = ATR interacting protein; DNA-PKcs = DNA-dependent protein kinase catalytic subunitNbs1 = Nijmegen breakage syndrome 1.

Supplementary Figure 4



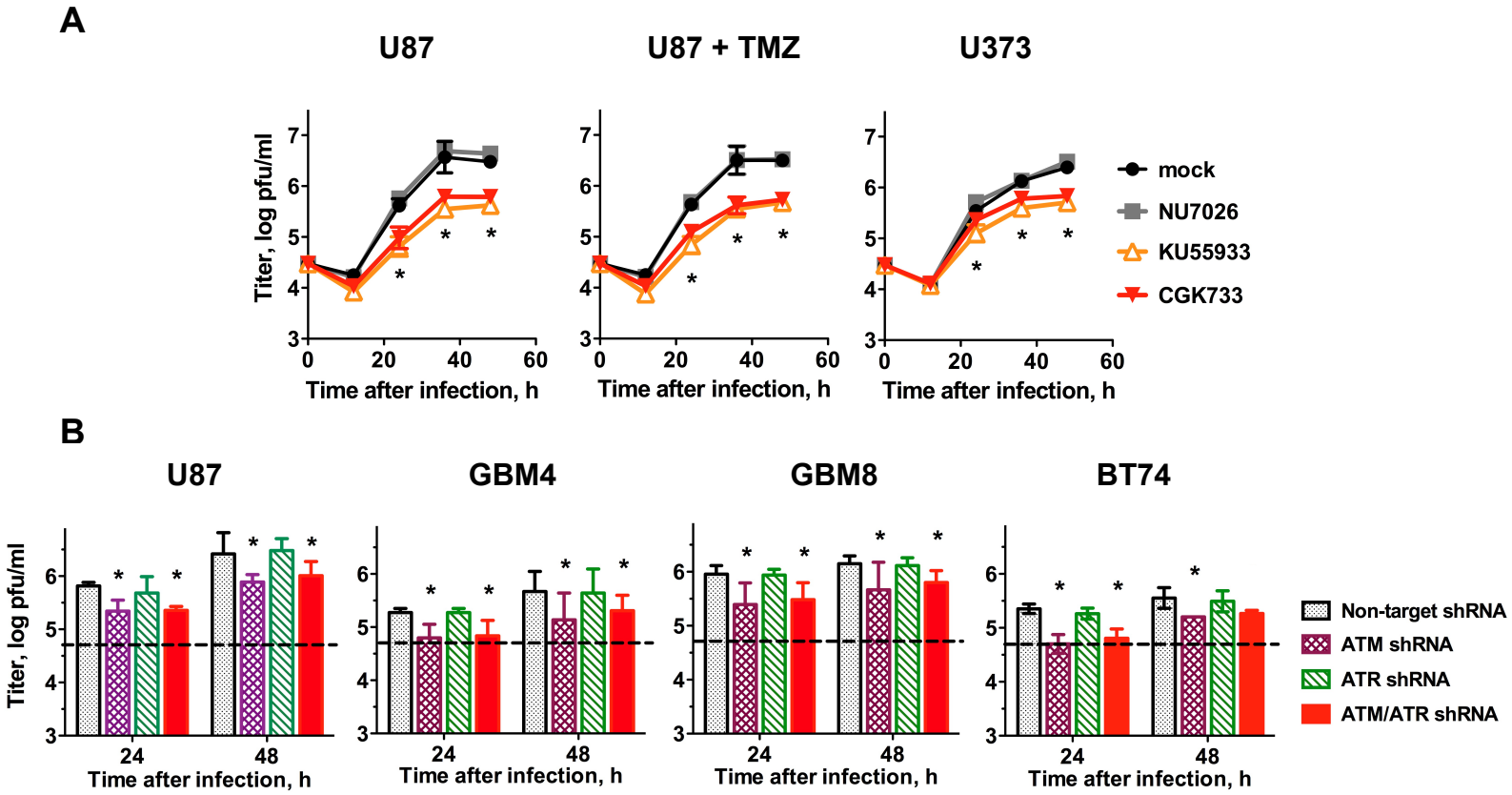
Supplementary Figure 4. Induction of γH2AX, and localization of DNA damage response proteins and G47Δ replication compartments in GSCs in vitro. A) γH2AX induction in GBM13 cells (MGMT-positive). Cells were mock-treated (M) or treated with TMZ (T, 200μM) and/or BG (B, 50μM) for 36 hours, and then mock-infected or infected with G47Δ (G, MOI=1), and 24 hours later, cells were fixed and processed for western blotting. Actin is the protein loading control. **B**) Accumulation of activated ATM (p-ATM) and Nbs1 (MRN complex) at G47Δ replication compartments. GBM13 (TMZ=200μM, BG=50μM) cells were fixed 6 hours after infection (MOI=5) and examined for immunofluorescence (DAPI, blue; p-ATM, green; ICP8 and Nbs1, red; merge, yellow). Scale bar=10μm. C) Nuclear accumulation of γH2AX in BT74 cells treated with PBS (mock), TMZ (200μM)+BG (50μM), G47Δ (MOI=1), or combination (γH2AX, green; DAPI, blue). Arrow points to γH2AX-negative nucleus. This is the same experiment illustrated in Figure 2,B. TMZ = temozolomide; GSCs = glioblastoma stem cells; MGMT = O^6-methylguanine-DNAmethyltransferase; BG = O^6-benzylguanine; MOI = multiplicity of infection; DAPI = 4', 6-diamidino2-phenylindole; ATM = ataxia telangiectasia mutated; p-ATM = phosphorylated ATM (Ser1981); Nbs1 = Nijmegen breakage syndrome 1.

Supplementary Figure 5



Supplementary Figure 5. Effect of ATM/ATR inhibition or knock-down on synergy in glioblastoma cells. A) The interactions between TMZ and G47Δ in GBM13 (**Left**) and U373 (**Right**) cells were examined in the presence of DNA-PKcs inhibitor NU7026, and ATM inhibitors KU55933 or CGK733. Data are shown as Fraction affected-Combination Index (CI) plot. **B**) Western blot for ATM and ATR from GSCs lentivirally-transduced with the indicated shRNAs (used in Figure 3C and Supplementary Figure 6B). **C)** The interactions between TMZ and G47Δ were examined in U87 cells lentivirally-transduced with shRNAs targeting ATM, ATR, ATM/ATR, or non-targeting. Data are shown as Fraction affected-Combination Index (CI) plot. **D**) Western blot for ATM and ATR from U87 lentivirally-transduced with the indicated shRNAs. CI < 1, CI = 1, and CI > 1 represent synergistic, additive, and antagonistic interactions, respectively. ATM = ataxia telangiectasia mutated; ATR = ataxia telangiectasia and Rad3-related; DNA-PKcs = DNA-dependent protein kinase catalytic subunit; GSCs = glioblastoma stem cells; shRNA = small hairpin RNA.

Supplementary Figure 6



Supplementary Figure 6. Effect of inhibitors or knock-down of ATM on G47Δ replication. A) G47Δ replication in the presence of DNA-PKcs inhibitor NU7026, or ATM inhibitors KU55933 or CGK733 with or without TMZ in U87 and U373 cells. Cells were infected with G47Δ at MOI=1.5 in the presence of NU7026, KU55933, or CGK733, and at the indicated times after infection, cells and media were harvested, and virus yields were determined by plaque assay on Vero cells. **Left:** U87 without TMZ, **Middle:** U87 with TMZ (50μM), **Right:** U373 without TMZ. Asterisk denotes statistically significant differences (unpaired *t*-test, two-tailed) in G47Δ titers between mock and KU55933 (for U87 *P* = .004, =.006, <.001 (difference=0.86, 95% CI = 0.73 to 0.98) at 24, 36, 48 h respectively; for U87+TMZ *P* = .003, =.005, <.001 (difference = 0.82, 95% CI = 0.62 to 1.02) at 24, 36, 48 h respectively; for U373 *P* = .01, =.001, =.002 (difference = 0.69, 95% CI = 0.44 to 0.94) at 24, 36, 48 h respectively) or CGK733 (for U87 *P* = .01, =.01, <.001 (difference = 0.69, 95% CI= 0.58 to 0.80) at 24, 36, 48 h respectively; for U87 + TMZ *P* = .002, =.008, <.001 (difference = 0.77, 95% CI = 0.61 to 0.93) at 24, 36, 48 h respectively; for U373 *P* = .001, <.001, <.001 (difference = 0.56, 95% CI = 0.40 to 0.73) at 24, 36, 48 h respectively) treated cells at indicated times after infection. Error bars represent 95% confidence intervals. **B**) G47Δ replication in GSCs and U87 cells lentivirally-transduced with shRNA targeting ATM, ATR, ATM/ATR, and non-targeted. Cells were treated as in **A**. Asterisk denotes statistically significant differences (unpaired *t*-test, two-tailed) in G47Δ titers between non-target shRNA-treated cells and ATM shRNA-treated (U87 24h: difference = 0.47, 95% CI = 0.33 to 0.61, *P* < .001; U87 48h: difference = 0.53, 95% CI = 0.26 to 0.80, *P* = .006; GBM4 24h: difference = 0.47, 95% CI = 0.30 to 0.65, *P* = .002; GBM4 48h: difference = 0.53, 95% CI = 0.12 to 0.94, *P* = .02; GBM8 24h: difference = 0.56, 95% CI = 0.28 to 0.84, *P* = .005; GBM8 48h: difference = 0.48, 95% CI = 0.14 to 0.82, *P* =.02; BT74 24h: difference = 0.65, 95% CI = 0.34 to 0.96, *P* = .004; BT74 48h: difference = 0.35, 95% CI= 0.04 to 0.66, *P* = .03) or ATM/ATR shRNA-treated (U87 24h: difference = 0.46, 95% CI = 0.39 to 0.52, *P* < .001; U87 48h: difference = 0.41, 95% CI = 0.10 to 0.72, *P* = .02; GBM4 24h: difference = 0.44, 95% CI = 0.24 to 0.63, *P* = .003; GBM4 48h: difference = 0.36, 95% CI = 0.05 to 0.67, *P* = .03; GBM8 24h: difference = 0.47, 95% CI = 0.25 to 0.70, *P* = .004; GBM8 48h: difference = 0.35, 95% CI = 0.18 to 0.52, *P* = .005; BT74 24h: difference = 0.55, 95% CI = 0.24 to 0.86, *P* = .008; BT74 48h: NS) cells at indicated times after infection. Error bars represent 95% confidence intervals. Dashed lines indicate the dose of input virus. From **left to right:** U87, GBM4, GBM8, BT74. TMZ = temozolomide; MOI = multiplicity of infection; GSCs = glioblastoma stem cells; DNA-PKcs = DNA-dependent protein kinase catalytic subunit; ATM = ataxia telangiectasia mutated; ATR = ataxia telangiectasia and Rad3-related; shRNA = short hairpin RNA; NS = not statistically significant.