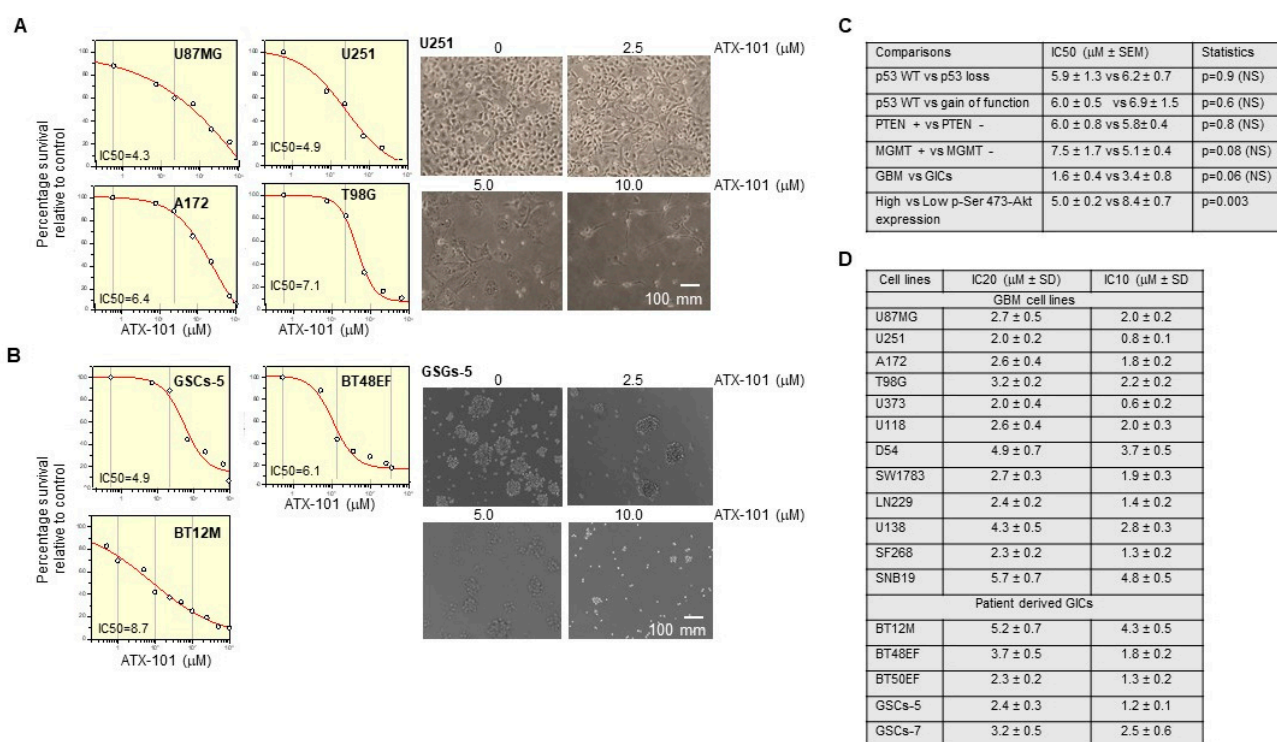


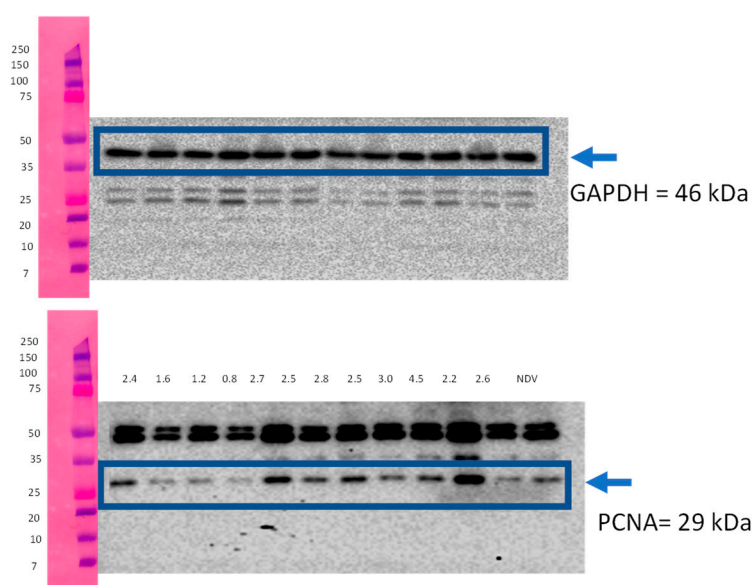
# ATX-101, a Peptide Targeting PCNA, Has Antitumor Efficacy Alone or in Combination with Radiotherapy in Murine Models of Human Glioblastoma

GRAVINA et al, 2021.

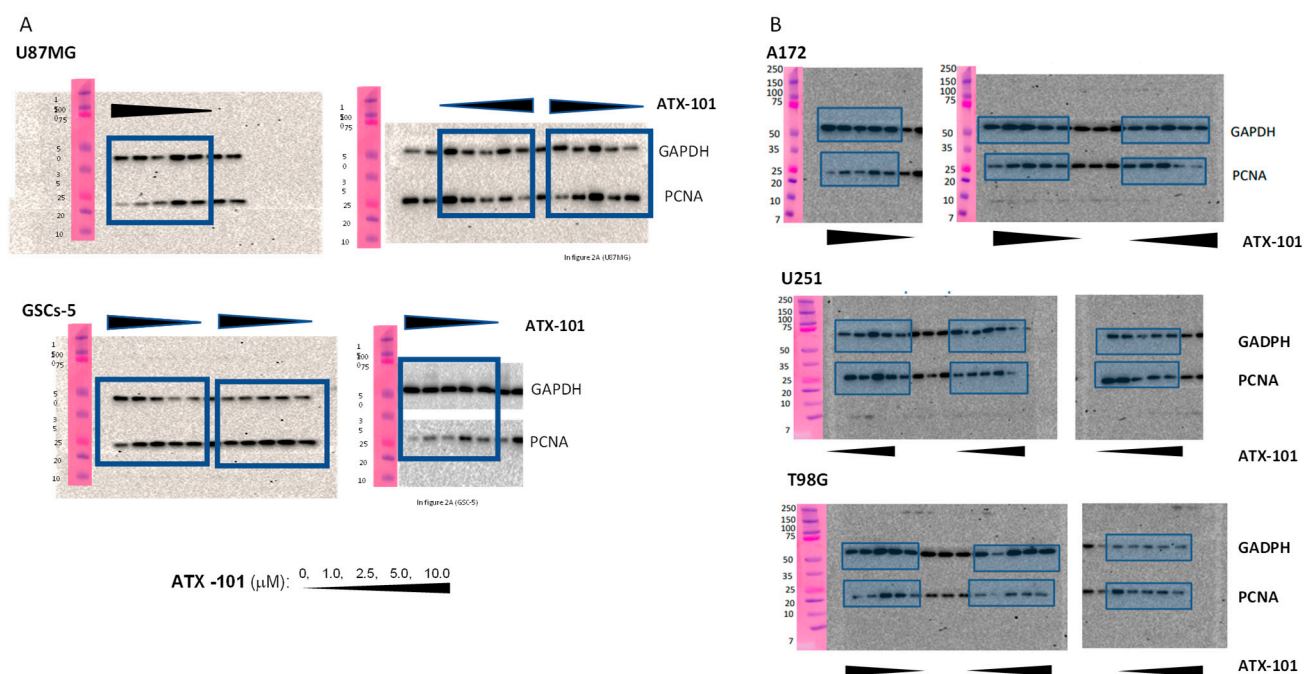
## Supplementary material



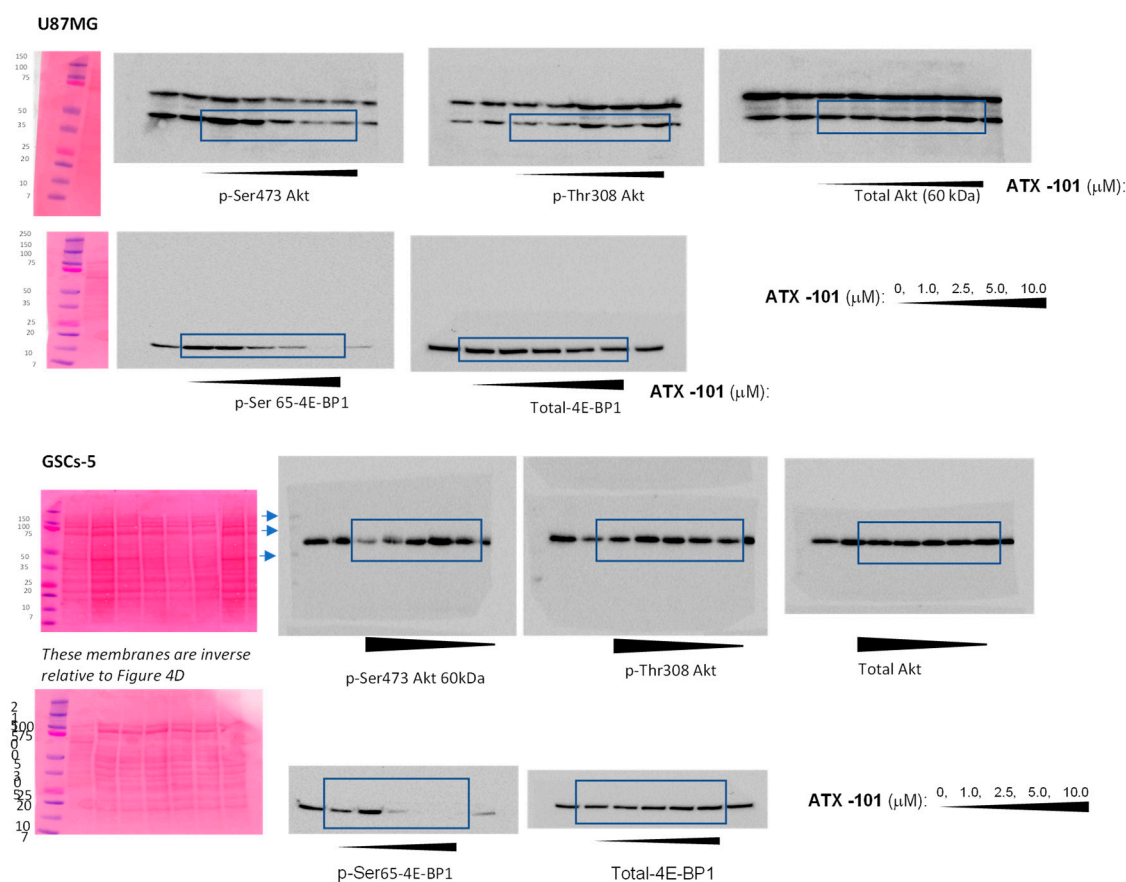
**Figure S1. Cell death induced by ATX-101 is independent of P53, PTEN and MGMT status in glioblastoma cell lines.** (A) Representative inhibition curves for ATX-101 treatment of U87MG, U251, A172 and T98G GMB cell lines (left panel). Representative images from U251 cell culture treated with different doses of ATX-101 (right panel). (B) Representative inhibition curves for ATX-101 treatment of the GICs GSCs-5, BT48EF and BT12M. Morphological appearance of GSCs-5 cultures treated with different doses of ATX-101 (right panel) (C) Comparisons between p53, PTEN, MGMT and p-Ser 473 Akt expression levels/status and IC50 values. Comparisons between IC50 values of GBM vs GIC cell lines. (D) IC20 and IC10 values calculated from survival curves. NS= not significant.



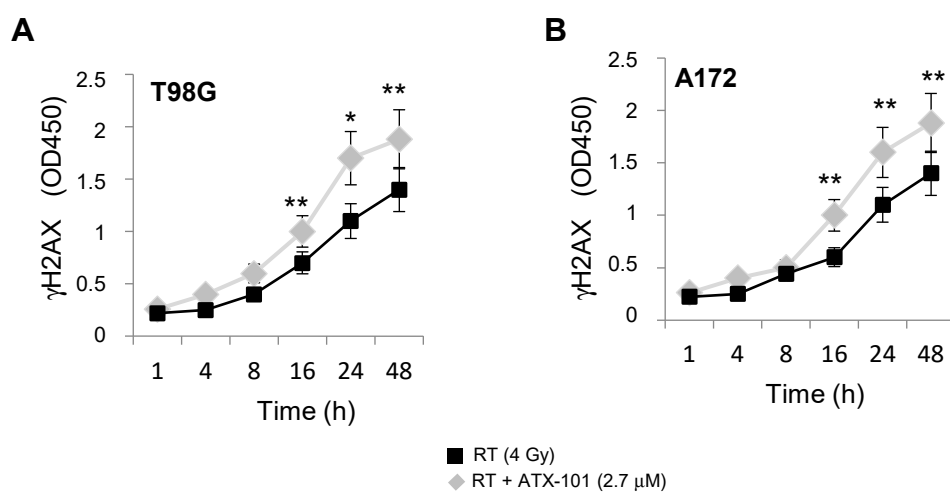
**Figure S2.** Uncropped Western blots shown in Figure 1C. For detailed description see figure legend in main manuscript.



**Figure S3.** Uncropped Western blots shown in Figure 2A and B. For detailed description see figure legend in main manuscript.

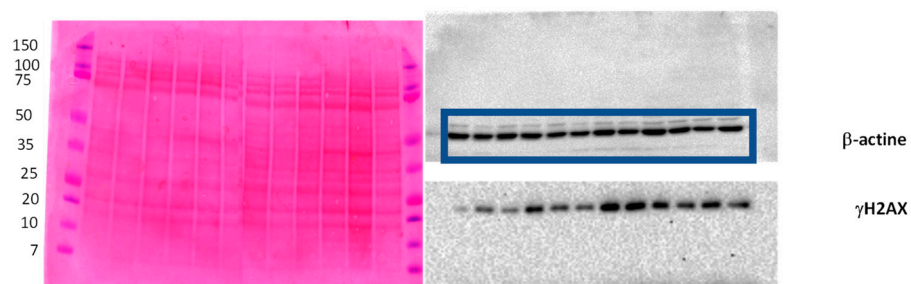


**Figure S4.** Uncropped Western blots shown in Figure 4D. For detailed description see figure legend in main manuscript.

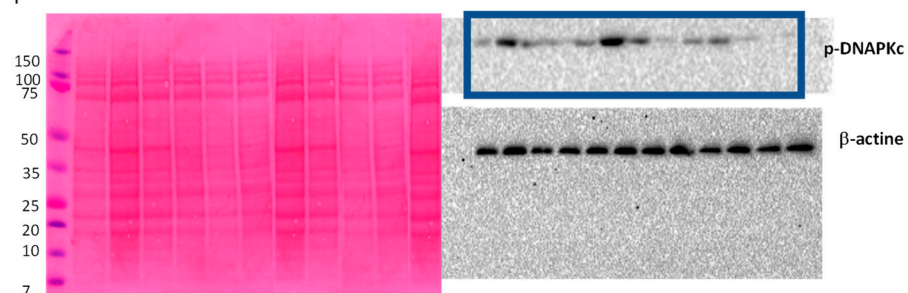


**Figure S5.** ATX-101 increases the levels of  $\gamma$ H2AX induced by RT. ELISA detection for  $\gamma$ H2AX in (A) T98G and (B) A172 glioma cell lines treated with ATX-101 (2.7  $\mu$ M) and RT (4 Gy) for 1- 48 h. Mean values of data from 3 cell extracts analysed in triplicates (total 9 determinations)  $\pm$  SD are shown. \* $p < 0.05$ , \*\*  $p < 0.01$ .

D



F



**Figure S6.** Uncropped Western blots shown in Figure 6D and F. For detailed description see figure legend in main manuscript.

**Table S1.** Characterization of GBM cell lines used.

Cell line	MGMT	TMZ	PTEN	p53	p-Ser 373-Akt	RT
U87MG	Methylated (-)	S	PTEN/mutated	p53 (+ WT)	High	S
U251	Methylated (-)	S	PTEN/null	p53/mutated	High	S
T98G	Non-methylated (+)	R	PTEN/null	p53/mutated	High	Moderately
A172	Methylated (-)	S	PTEN (+WT)	p53 (+WT)	Low	Moderately
D54	Methylated (-)	S	PTEN (+WT)	p53 (+WT)	Low	R
SW1783	Methylated (-)	S	PTEN (+WT)	p53 (+WT)	Low	R
U118	Non-methylated (+)	R	PTEN/null	p53 (+WT)	High	R
U373	Methylated (-)	S	PTEN/null	p53/mutated	High	S
SNB19	Non-methylated (+)	R	PTEN mutated	p53 (+WT)	High	Moderately
L229	Methylated (-)	S	PTEN (+WT)	p53/mutated	Low	S
SF268	Non-methylated (+)	R	PTEN/mutated	p53/mutated	High	Moderately
U138	Non-methylated (+)	R	PTEN (+WT)	p53/mutated	low	R

**Table S2.** Weights of mice and tumours in the subcutaneous xenograft model.

Data shown in Figure 7B.

Cells, Treatments	Weight of mice (mean $\pm$ SEM)	Tumour weight (mean $\pm$ SEM)
U87MG, Vehicle	25.0 $\pm$ 0.3	987 $\pm$ 71
U87MG, ATX-101	25.0 $\pm$ 0.2	385 $\pm$ 55
U87MG, RT	24.8 $\pm$ 0.5	775 $\pm$ 40
U87MG, RT + ATX-101	24.5 $\pm$ 0.8	166 $\pm$ 24
T98G, Vehicle	24.5 $\pm$ 0.3	1015 $\pm$ 61
T98G, ATX-101	26.0 $\pm$ 0.2	655 $\pm$ 40
T98G, RT	24.8 $\pm$ 0.5	710 $\pm$ 46
T98G, RT + ATX-101	24.5 $\pm$ 0.3	346 $\pm$ 22

**Table S3.** Radio-sensitization of ATX-101 on U87MG and T98G subcutaneous xenografts. Statistical analyses of Kaplan Meyer curves shown in Figure 7.

	U87MG				T98G			
Comparisons	HR	95% CI	Statistics	Combina- tion index, CI	HR	95% CI	Statistics	Combina- tion index, CI
Vehicle vs ATX-101	4.3	1.5 - 15.4	p<0.001	ATX+RT CI=1.05 (Additive)	3.4	1.2 - 11.1	p<0.001	ATX+RT CI=0.93 (sub- additive)
Vehicle vs RT	2.2	1.0 - 6.6	p<0.05		3.0	1.0 - 9.7	p<0.001	
RT vs ATX-101	3.8	1.3 - 11.2	p<0.005		1.5	0.6 - 3.9	NS	
Vehicle vs ATX-101 + RT	6.8	1.9-- 21.1	p<0.0001		5.9	1.4 - 17.7	p<0.0001	
ATX-101 vs ATX-101 + RT	3.6	1.2 - 9.17	p<0.01		3.0	1.0 - 8.5	p<0.01	
RT vs ATX-101 + RT	4.6	1.5 - 14.2	p<0.001		2.3	0.9 - 6.4	p<0.05	

NS= not significant.

**Table S4.** Statistical analyses for disease-free survival (DFS) and overall survival OS (A) and HR ratios (B) for animals treated with ATX-101 with or without RT for the orthotopic brain tumour model using luciferase tagged U87MG cells. Kaplan Meyer curves shown in Figure 8.

(A)

	Disease-free survival (DFS)			Overall survival (OS)		
Treatment	Range (days)	Mean $\pm$ SEM	Statistics	Range (days)	Mean $\pm$ SEM	Statistics
Vehicle	15-35	21.5 $\pm$ 2.4		40-70	56.0 $\pm$ 3.0	
ATX-101	30-120	53.0 $\pm$ 8.8	p<0.01 vs Vehicle	80-180	102.7 $\pm$ 10.7	p<0.01 vs Vehicle
RT	25-40	330 $\pm$ 4.8	p<0.01 vs Vehicle p<0.05 vs ATX-101	45-90	69.0 $\pm$ 4.0	p<0.05 vs Vehicle p<0.01 vs ATX-101
ATX-101 + RT	50-250	114.5 $\pm$ 24.7	p<0.01 vs Vehicle p<0.01 vs ATX-101 p<0.01 vs RT	88-250	157.3 $\pm$ 22.5	p<0.01 vs Vehicle p<0.05 vs ATX-101 p<0.01 vs RT

(B)

	Disease-free survival (DFS)				Overall survival (OS)			
Comparison	HR	95% CI	Statistics	Combination index, CI	HR	95% CI	Statistics	Combination index, CI
Vehicle vs ATX-101	3.3	1.2-11.4	p<0.01	ATX+RT CI=1.02 Additive	4.9	1.5-15.1	p<0.01	ATX+RT CI=1.06 Additive
Vehicle vs RT	2.4	0.9-6.3	p<0.01		2.4	1.0-7.0	p<0.01	
RT vs ATX-101	2.5	0.9-6.5	p<0.01		3.3	1.2-9.4	p<0.01	
Vehicle vs ATX-101 + RT	5.5	2.0-13.9	p<0.01		7.7	2.6-16.9	p<0.01	
ATX-101 vs ATX-101 + RT	4.9	1.5-15.0	p<0.01		4.0	1.4-10.0	p<0.01	
RT vs ATX-101 + RT	3.0	1.1-8.3	p<0.01		5.1	1.6-15.6	p<0.01	

**Table S5.** Statistical analyses for DFS and OS (A) and HR ratios (B) for animals treated with ATX-101 with or without RT for the orthotopic brain tumour model using luciferase tagged GSGs-5 cells. Kaplan Meyer curves shown in Figure 8.

(A)

Treatment	Disease-free survival (DFS)			Overall survival (OS)		
	Range (days)	Mean $\pm$ SEM	Statistics	Range (days)	Mean $\pm$ SEM	Statistics
Vehicle	35-65	46.0 $\pm$ 3.0		40-70	79.5 $\pm$ 3.6	
ATX-101	60-85	73.5 $\pm$ 2.6	p<0.001 vs Vehicle	94-145	74.5 $\pm$ 4.6	NS vs Vehicle
RT	25-60	37.0 $\pm$ 4.0	NS vs Vehicle p<0.01 vs ATX-101	45-90	69.0 $\pm$ 4.0	p<0.05 vs Vehicle p<0.01 vs ATX-101
ATX-101 + RT	50-250	117.5 $\pm$ 24.3	p<0.01 vs Vehicle p<0.01 vs ATX-101 p<0.01 vs RT	88-250	170.3 $\pm$ 22.4	p<0.01 vs Vehicle p<0.01 vs RT p<0.01 vs ATX-101

NS= not significant.

(B)

Comparison	Disease-free survival (DFS)				Overall survival (OS)			
	HR	95% CI	Statistics	Combination index, CI	HR	95% CI	Statistics	Combination index, CI
Vehicle vs ATX-101	4.0	1.3-11.1	p<0.01	ATX+RT CI=0.97 Sub-Additive	4.4	1.4-13.7	p<0.01	ATX+RT CI=1.11 Synergistic
Vehicle vs RT	0.6	0.3-1.5	NS		0.8	0.3-2.0	NS	
RT vs ATX-101	4.2	1.3-16.7	p<0.01		4.7	1.48-13.57	p<0.01	
Vehicle vs ATX-101 + RT	4.5	1.3-11.4	p<0.01		5.8	1.6-14.9	p<0.01	
ATX-101 vs ATX-101 + RT	1.9	0.7-4.9	NS		2.4	0.9-6.3	p<0.05	
RT vs ATX-101 + RT	3.9	1.3-11.4	p<0.01		4.5	1.5-13.6	p<0.01	

NS= not significant.