

**Figure W1.** Changes of intracellular ATP in glioblastoma cells exposed to serial dilutions of NVP-BEZ235 for 24 and 48 hours. ATP content was measured by standard luciferase bioluminescence assay. Quadruplicate data derived from at least three independent experiments were averaged, normalized against non-treated controls (DMSO), and analyzed using the standard 4PLM (Equation 1) to generate dose-response curves. Error bars indicate SD values. Depicted are the calculated IC<sub>50</sub> concentrations.

**Table W1.** PEs and Radiosensitivity Parameters of Control, Irradiated, and/or Drug-Treated Tumor Cell Lines under Two Different Drug-IR Schedules.

Cell Line	Treatment		PE*	SF2 <sup>†</sup>	<i>D</i> <sub>10</sub> <sup>‡</sup>	<i>I</i> <sub>10</sub> ( <i>D</i> <sub>10</sub> Control/ <i>D</i> <sub>10</sub> NVP-BEZ235)
DK-MG	Schedule I	Control	0.22 ± 0.06	0.63 ± 0.12	5.73 ± 0.93	1.08 ± 0.18
		BEZ235	0.18 ± 0.04	0.58 ± 0.12	5.33 ± 0.73	
	Schedule II	Control	0.17 ± 0.09	0.67 ± 0.09	7.93 ± 0.54	1.92 ± 0.16
		BEZ235	0.16 ± 0.08	0.38 ± 0.05	4.15 ± 0.25	
U87-MG	Schedule I	Control	0.23 ± 0.15	0.54 ± 0.10	5.82 ± 0.35	0.89 ± 0.12
		BEZ235	0.18 ± 0.07	0.58 ± 0.04	6.46 ± 0.50	
	Schedule II	Control	0.20 ± 0.08	0.62 ± 0.02	8.52 ± 0.39	2.1 ± 0.14
		BEZ235	0.16 ± 0.09	0.36 ± 0.05	4.06 ± 0.31	
GaMG	Schedule I	Control	0.17 ± 0.04	0.74 ± 0.09	7.69 ± 0.80	1.00 ± 0.05
		BEZ235	0.14 ± 0.02	0.79 ± 0.04	7.69 ± 0.61	
	Schedule II	Control	0.17 ± 0.06	0.80 ± 0.08	10.21 ± 2.32	2.21 ± 0.48
		BEZ235	0.17 ± 0.06	0.46 ± 0.08	4.64 ± 0.51	
U373	Schedule I	Control	0.38 ± 0.18	0.64 ± 0.12	6.01 ± 0.50	0.99 ± 0.11
		BEZ235	0.35 ± 0.02	0.59 ± 0.02	6.15 ± 0.83	
	Schedule II	Control	0.29 ± 0.07	0.74 ± 0.08	9.01 ± 0.61	2.51 ± 0.58
		BEZ235	0.30 ± 0.07	0.30 ± 0.09	3.81 ± 1.14	

Data are presented as means (±SD) from at least four independent experiments. For detailed description, see legend to Figure 2.

\*PE, plating efficiency.

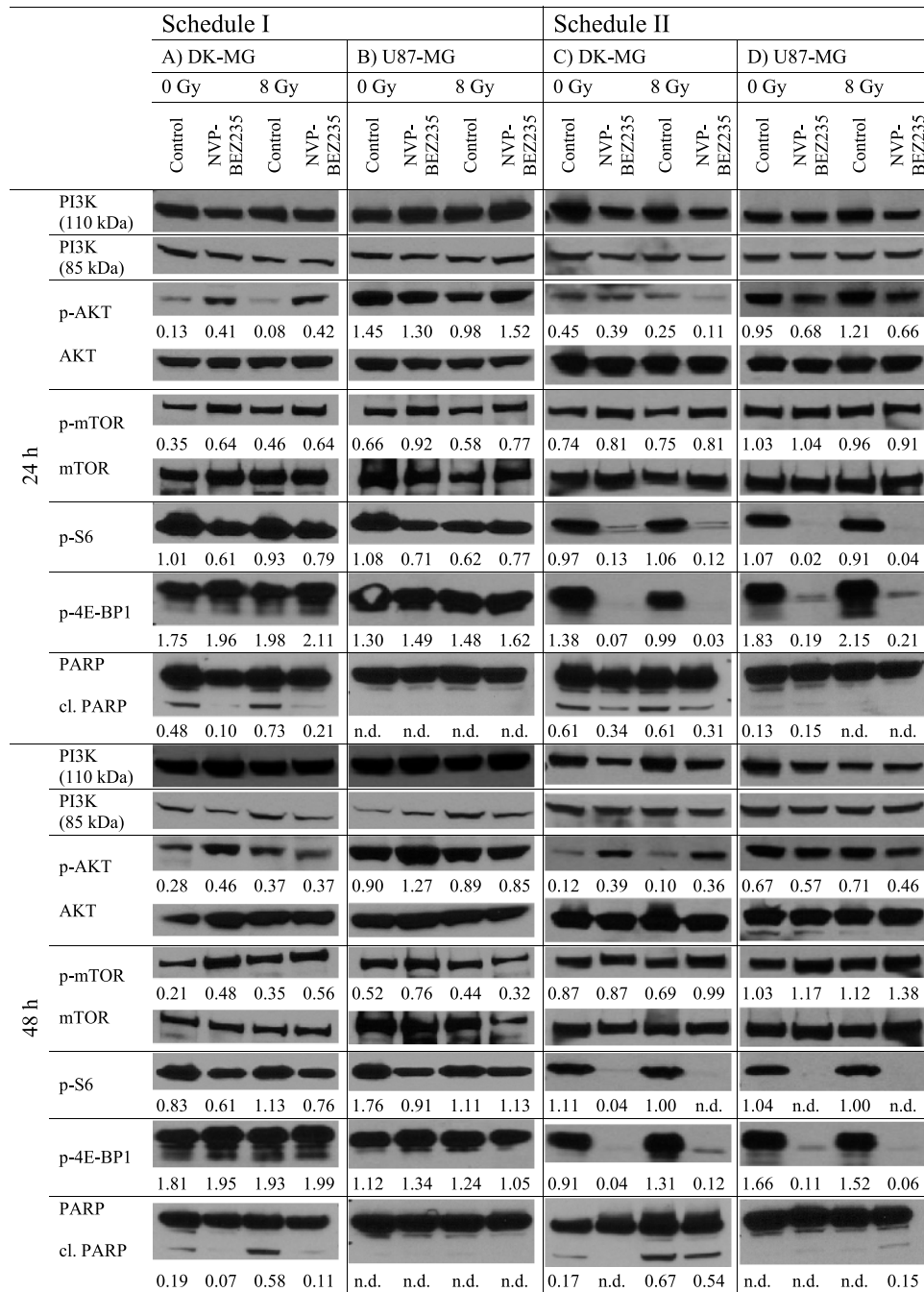
<sup>†</sup>SF2, surviving fraction at 2 Gy.

<sup>‡</sup>*D*<sub>10</sub>, radiation dose yielding 10% survival; *I*<sub>10</sub>, growth inhibition factor was calculated as (*D*<sub>10</sub> control)/*D*<sub>10</sub> + inhibitor).

§  $\Delta \leq .05$ .

	Schedule I				Schedule II											
	A) GaMG		B) U373		C) GaMG		D) U373									
	0 Gy	8 Gy	0 Gy	8 Gy	0 Gy	8 Gy	0 Gy	8 Gy								
	Control	NVP- BEZ235	Control	NVP- BEZ235	Control	NVP- BEZ235	Control	NVP- BEZ235								
PTEN																
PI3K (110 kDa)																
PI3K (85 kDa)																
p-AKT	0.18	0.51	0.10	0.53	0.28	0.46	0.22	0.61	0.37	0.13	0.29	0.05	0.74	0.19	0.57	0.10
AKT																
p-mTOR	0.45	0.79	0.57	0.74	1.01	1.09	1.10	1.07	0.65	0.46	0.49	0.50	0.73	0.45	0.65	0.34
mTOR																
p-S6	1.81	0.10	2.23	0.35	2.50	0.99	2.67	1.03	0.93	1.04	0.96	1.28	1.06	1.16	1.12	1.22
p-4E-BP1	0.58	0.63	0.96	0.81	1.14	0.92	0.96	0.70	1.91	0.40	1.78	0.12	2.11	0.30	2.31	0.22
PARP cl. PARP	0.10	0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.08	n.d.	0.07	0.08	n.d.	n.d.	n.d.	n.d.

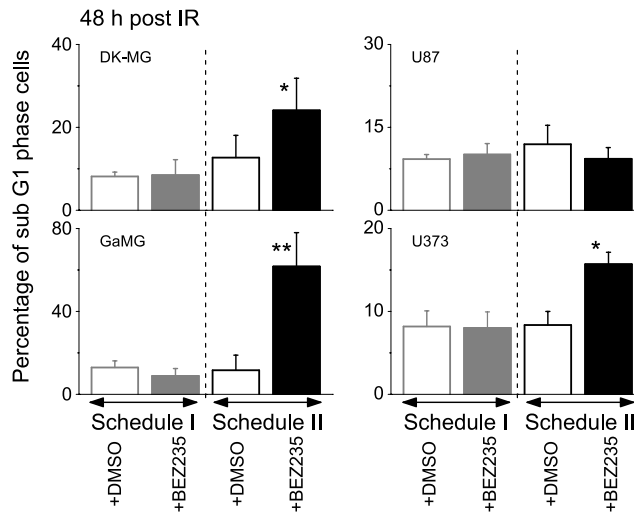
**Figure W2.** Effects of NVP-BEZ235 and IR on the expression levels of marker proteins in GaMG (A, C) and U373 (B, D) cell lines detected 30 minutes after IR. Cells were treated with NVP-BEZ235 and IR either in schedule I (left side) or schedule II (right side). For details, see legend to Figure 3. n.d. indicates not determined.



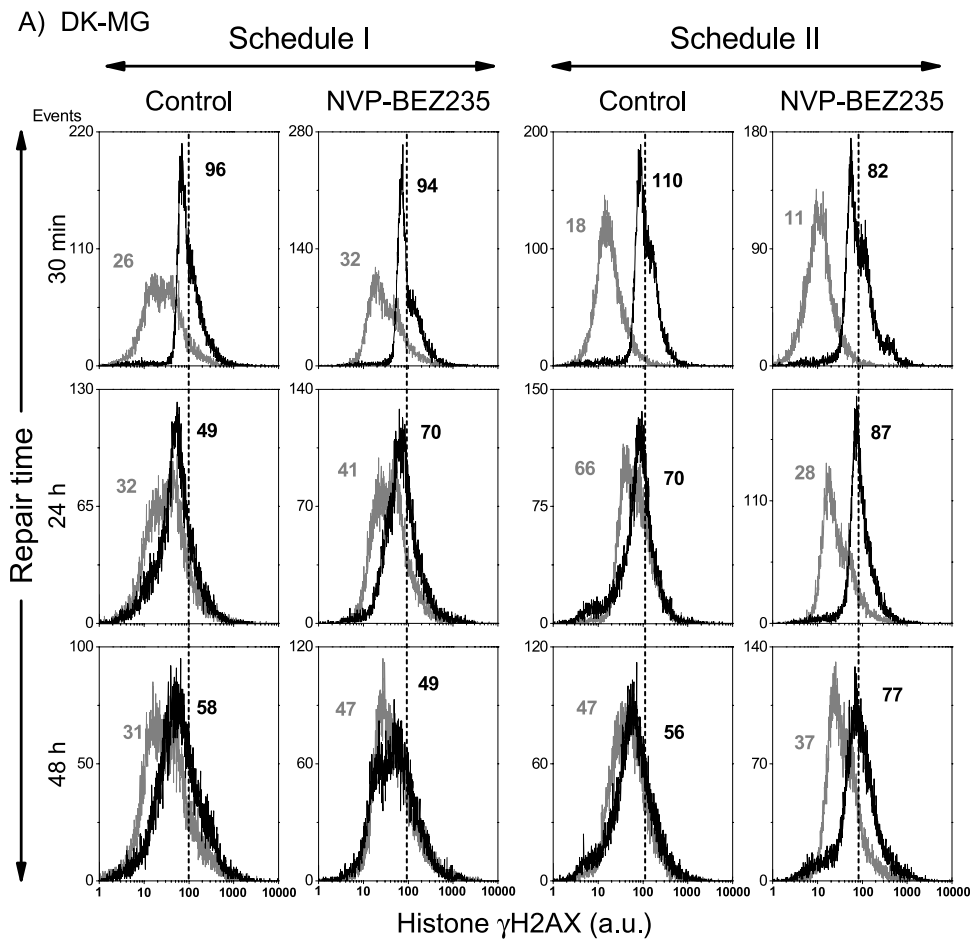
**Figure W3.** Effects of NVP-BEZ235 and IR on the expression levels of marker proteins in DK-MG (A, C) and U87-MG (B, D) cell lines detected 24 and 48 hours after IR. Cells were treated with NVP-BEZ235 and IR either in schedule I (left side) or schedule II (right side). For details, see legend to Figure 3.

		Schedule I								Schedule II							
		A) GaMG				B) U373				C) GaMG				D) U373			
		0 Gy		8 Gy		0 Gy		8 Gy		0 Gy		8 Gy		0 Gy		8 Gy	
		Control	NVP-BEZ235	Control	NVP-BEZ235	Control	NVP-BEZ235	Control	NVP-BEZ235	Control	NVP-BEZ235	Control	NVP-BEZ235	Control	NVP-BEZ235	Control	NVP-BEZ235
24 h	PI3K (110 kDa)	[band]		[band]		[band]		[band]		[band]		[band]		[band]		[band]	
	PI3K (85 kDa)	[band]		[band]		[band]		[band]		[band]		[band]		[band]		[band]	
	p-AKT	0.17	0.47	0.46	0.59	1.10	1.66	0.90	1.60	0.34	0.17	0.26	0.11	0.91	0.82	0.69	0.96
	AKT	[band]		[band]		[band]		[band]		[band]		[band]		[band]		[band]	
	p-mTOR	0.96	0.74	0.74	0.71	0.83	1.26	1.10	1.25	0.60	0.64	0.56	0.57	0.75	0.63	0.70	0.71
	mTOR	[band]		[band]		[band]		[band]		[band]		[band]		[band]		[band]	
	p-S6	2.33	1.19	1.77	0.52	2.24	1.08	2.41	1.73	2.00	n.d.	0.94	n.d.	1.77	n.d.	0.70	n.d.
	p-4E-BP1	1.09	1.07	0.91	1.08	1.44	1.29	1.24	1.34	1.65	0.06	1.42	0.07	2.69	0.27	2.32	0.17
	PARP	[band]		[band]		[band]		[band]		[band]		[band]		[band]		[band]	
	cl. PARP	0.10	n.d.	0.39	n.d.	0.04	0.09	0.27	0.24	n.d.	n.d.	0.32	1.72	0.26	0.36	0.50	0.57
48 h	PI3K (110 kDa)	[band]		[band]		[band]		[band]		[band]		[band]		[band]		[band]	
	PI3K (85 kDa)	[band]		[band]		[band]		[band]		[band]		[band]		[band]		[band]	
	p-AKT	0.43	0.39	0.63	0.43	1.28	1.23	1.18	1.29	0.24	0.15	0.32	0.14	n.d.	1.04	0.53	0.87
	AKT	[band]		[band]		[band]		[band]		[band]		[band]		[band]		[band]	
	p-mTOR	0.75	0.76	0.60	0.68	0.87	1.04	0.87	0.81	0.19	0.54	0.24	0.68	0.17	0.98	0.65	0.90
	mTOR	[band]		[band]		[band]		[band]		[band]		[band]		[band]		[band]	
	p-S6	1.12	1.13	1.53	1.16	1.45	0.87	1.97	1.25	1.08	n.d.	1.78	n.d.	1.69	n.d.	1.46	n.d.
	p-4E-BP1	1.08	0.90	0.98	1.01	0.98	1.14	1.20	1.43	1.73	0.08	1.57	0.12	3.91	0.36	2.71	0.14
	PARP	[band]		[band]		[band]		[band]		[band]		[band]		[band]		[band]	
	cl. PARP	0.42	0.34	0.88	0.85	n.d.	n.d.	0.24	n.d.	0.38	0.45	0.58	1.55	n.d.	n.d.	0.35	0.69

**Figure W4.** Effects of NVP-BEZ235 and IR on the expression levels of marker proteins in GaMG (A, C) and U373 (B, D) cell lines detected 24 and 48 hours after IR. Cells were treated with NVP-BEZ235 and IR either in schedule I (left side) or schedule II (right side). For details, see legend to Figure 3.

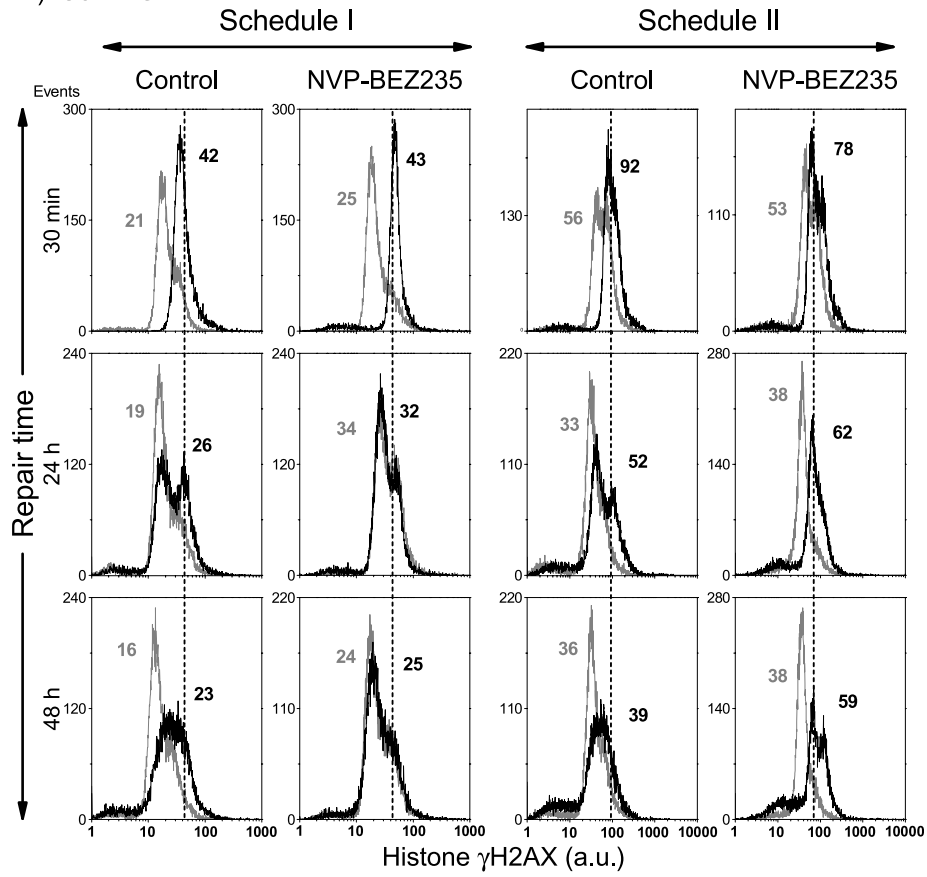


**Figure W5.** Effects of NVP-BE235 and IR on the late-stage apoptosis (subG<sub>1</sub> cells). Cells were treated with NVP-BE235 and IR either in schedule I or schedule II. For details, see legend to Figure 4.



**Figure W6.** (A) Effects of NVP-BE235 and IR on the induction and repair of DNA damage in DK-MG (A) cell line. Cells were treated with NVP-BE235 and IR either in schedule I (left side) or schedule II (right side). For details, see legend to Figure 5. (B) Effects of NVP-BE235 and IR on the induction and repair of DNA damage in U87-MG cell line. Cells were treated with NVP-BE235 and IR either in schedule I (left side) or schedule II (right side). For details, see legend to Figure 5. (C) Effects of NVP-BE235 and IR on the induction and repair of DNA damage in U373 cell line. Cells were treated with NVP-BE235 and IR either in schedule I (left side) or schedule II (right side). For details, see legend to Figure 5.

B) U87-MG



C) U373

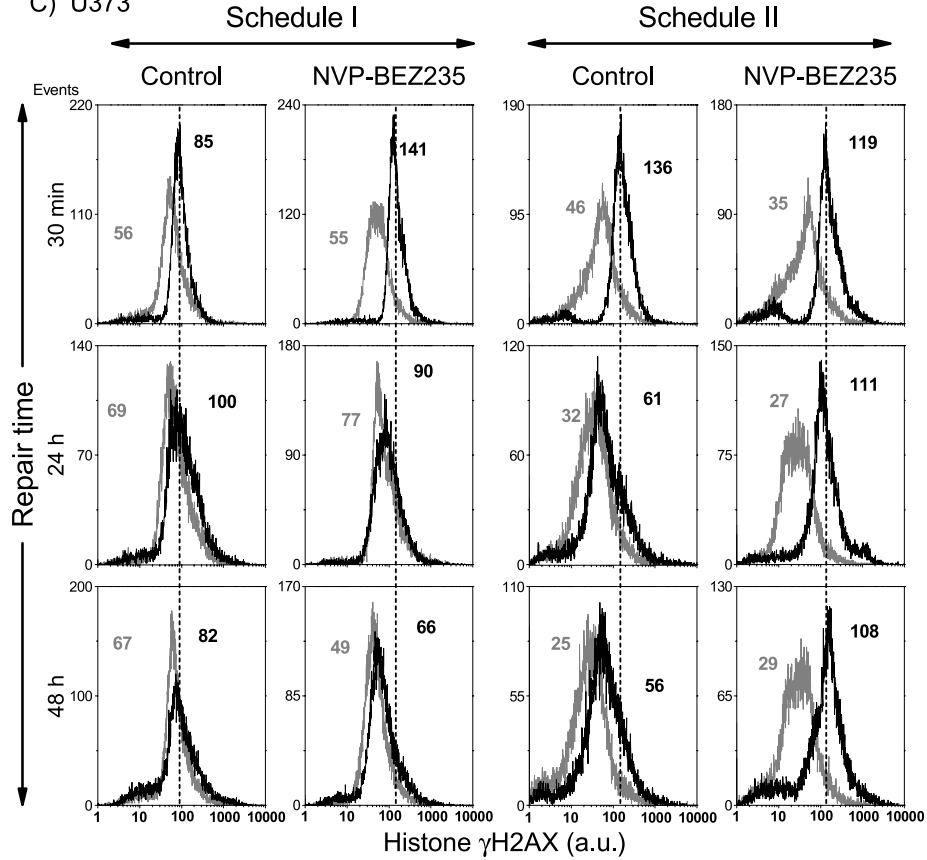


Figure W6. (continued).

**Table W2.** Cell Cycle Phase Distribution in Tumor Cells Treated with NVP-BEZ235 and IR according to Different Schedules.

Cell Line	Treatment Modality			G <sub>0</sub> /G <sub>1</sub> (%)	S (%)	G <sub>2</sub> /M (%)	G <sub>2</sub> /G <sub>1</sub>
DK-MG	Schedule I	0 Gy	Control	53 ± 4	22 ± 3	26 ± 3	0.49
			NVP-BEZ235	73 ± 2	9 ± 2	18 ± 2	0.25
		8 Gy	Control	54 ± 3	21 ± 3	25 ± 3	0.46
			NVP-BEZ235	74 ± 2	10 ± 2	17 ± 2	0.23
	Schedule II	0 Gy	Control	46 ± 4	28 ± 3	26 ± 3	0.57
			NVP-BEZ235	45 ± 4	29 ± 5	26 ± 3	0.58
	8 Gy	Control	46 ± 4	28 ± 3	26 ± 4	0.57	
		NVP-BEZ235	46 ± 5	28 ± 3	26 ± 4	0.57	
U87-MG	Schedule I	0 Gy	Control	70 ± 3	20 ± 3	10 ± 2	0.14
			NVP-BEZ235	90 ± 4	6 ± 2	5 ± 2	0.06
		8 Gy	Control	71 ± 3	19 ± 2	10 ± 4	0.14
			NVP-BEZ235	89 ± 3	6 ± 1	5 ± 2	0.06
	Schedule II	0 Gy	Control	51 ± 8	28 ± 3	22 ± 6	0.43
			NVP-BEZ235	51 ± 9	28 ± 3	21 ± 6	0.41
	8 Gy	Control	49 ± 9	29 ± 4	22 ± 7	0.45	
		NVP-BEZ235	50 ± 9	28 ± 4	22 ± 6	0.44	
GaMG	Schedule I	0 Gy	Control	60 ± 6	28 ± 4	12 ± 4	0.20
			NVP-BEZ235	80 ± 3	11 ± 4	9 ± 4	0.11
		8 Gy	Control	61 ± 7	27 ± 5	12 ± 5	0.20
			NVP-BEZ235	79 ± 3	13 ± 5	8 ± 3	0.10
	Schedule II	0 Gy	Control	40 ± 12	34 ± 11	26 ± 16	0.65
			NVP-BEZ235	41 ± 11	36 ± 11	23 ± 16	0.56
	8 Gy	Control	40 ± 12	35 ± 12	25 ± 16	0.63	
		NVP-BEZ235	42 ± 11	33 ± 16	24 ± 18	0.57	
U373	Schedule I	0 Gy	Control	67 ± 6	17 ± 3	16 ± 5	0.25
			NVP-BEZ235	65 ± 11	19 ± 7	16 ± 4	0.25
		8 Gy	Control	65 ± 5	18 ± 3	17 ± 5	0.26
			NVP-BEZ235	62 ± 10	21 ± 6	17 ± 5	0.27
	Schedule II	0 Gy	Control	36 ± 8	37 ± 13	28 ± 10	0.78
			NVP-BEZ235	38 ± 10	37 ± 13	25 ± 11	0.66
	8 Gy	Control	39 ± 9	36 ± 13	25 ± 9	0.64	
		NVP-BEZ235	38 ± 10	37 ± 13	24 ± 11	0.63	

Thirty minutes after IR, cells were fixed, permeabilized, stained with PI, and analyzed for DNA content by flow cytometry. Data are presented as means (±SD) from at least four independent experiments. For detailed description, see legend to Figure 6.

**Table W3.** Cell Cycle Phase Distribution in Tumor Cells Treated with NVP-BEZ235 and IR according to Different Schedules.

Cell Line	Treatment Modality			G <sub>0</sub> /G <sub>1</sub> (%)	S (%)	G <sub>2</sub> /M (%)	G <sub>2</sub> /G <sub>1</sub>
DK-MG	Schedule I	0 Gy	Control	49 ± 3	24 ± 4	27 ± 3	0.55
			NVP-BEZ235	49 ± 9	26 ± 4	25 ± 6	0.51
	Schedule II	8 Gy	Control	21 ± 4	8 ± 1	71 ± 4	3.38
			NVP-BEZ235	22 ± 12	31 ± 4	47 ± 12	2.14
		0 Gy	Control	55 ± 6	22 ± 2	23 ± 6	0.42
			NVP-BEZ235	74 ± 6	9 ± 2	17 ± 6	0.23
U87-MG	Schedule I	8 Gy	Control	29 ± 13	9 ± 3	63 ± 16	2.17
			NVP-BEZ235	27 ± 16	11 ± 3	62 ± 13	2.30
	Schedule II	0 Gy	Control	71 ± 3	18 ± 2	10 ± 1	0.14
			NVP-BEZ235	67 ± 3	20 ± 4	14 ± 2	0.21
		8 Gy	Control	58 ± 6	10 ± 1	32 ± 5	0.55
			NVP-BEZ235	56 ± 8	19 ± 4	25 ± 6	0.45
GaMG	Schedule I	0 Gy	Control	68 ± 8	20 ± 2	12 ± 6	0.18
			NVP-BEZ235	85 ± 7	9 ± 2	6 ± 6	0.07
	Schedule II	8 Gy	Control	59 ± 14	9 ± 1	33 ± 15	0.56
			NVP-BEZ235	58 ± 17	4 ± 3	37 ± 15	0.64
		0 Gy	Control	73 ± 7	18 ± 5	9 ± 3	0.12
			NVP-BEZ235	50 ± 14	26 ± 5	24 ± 4	0.48
U373	Schedule I	8 Gy	Control	65 ± 6	17 ± 4	17 ± 4	0.26
			NVP-BEZ235	27 ± 9	22 ± 5	51 ± 9	1.89
	Schedule II	0 Gy	Control	62 ± 4	28 ± 3	11 ± 3	0.18
			NVP-BEZ235	79 ± 6	12 ± 5	9 ± 2	0.11
		8 Gy	Control	55 ± 12	26 ± 4	19 ± 6	0.35
			NVP-BEZ235	40 ± 8	12 ± 4	47 ± 8	1.18
U373	Schedule I	0 Gy	Control	67 ± 7	12 ± 2	21 ± 5	0.31
			NVP-BEZ235	62 ± 5	15 ± 2	23 ± 3	0.37
	Schedule II	8 Gy	Control	57 ± 8	8 ± 3	35 ± 6	0.61
			NVP-BEZ235	37 ± 8	14 ± 3	49 ± 8	1.32
		0 Gy	Control	64 ± 5	18 ± 4	19 ± 3	0.30
			NVP-BEZ235	59 ± 5	24 ± 3	17 ± 2	0.29
		8 Gy	Control	60 ± 5	10 ± 2	30 ± 6	0.50
			NVP-BEZ235	24 ± 27	12 ± 5	64 ± 27	2.67

Twenty-four hours after IR, cells were fixed, permeabilized, stained with PI, and analyzed for DNA content by flow cytometry. Data are presented as means (±SD) from at least four independent experiments. For detailed description, see legend to Figure 6.



**Table W4.** Cell Cycle Phase Distribution in Tumor Cells Treated with NVP-BEZ235 and IR according to Different Schedules.

Cell Line	Treatment Modality			G <sub>0</sub> /G <sub>1</sub> (%)	S (%)	G <sub>2</sub> /M (%)	G <sub>2</sub> /G <sub>1</sub>
DK-MG	Schedule I	0 Gy	Control	56 ± 4	20 ± 2	24 ± 3	0.43
			NVP-BEZ235	66 ± 7	13 ± 5	20 ± 4	0.30
		8 Gy	Control	39 ± 4	24 ± 2	37 ± 3	0.95
			NVP-BEZ235	53 ± 6	7 ± 3	41 ± 6	0.77
	Schedule II	0 Gy	Control	53 ± 4	23 ± 7	24 ± 6	0.45
			NVP-BEZ235	72 ± 3	10 ± 4	18 ± 2	0.25
		8 Gy	Control	37 ± 3	23 ± 3	40 ± 3	1.08
			NVP-BEZ235	26 ± 9	10 ± 6	64 ± 8	2.46
U87-MG	Schedule I	0 Gy	Control	71 ± 3	19 ± 2	10 ± 2	0.14
			NVP-BEZ235	70 ± 3	19 ± 2	11 ± 3	0.16
		8 Gy	Control	59 ± 2	17 ± 2	24 ± 2	0.41
			NVP-BEZ235	68 ± 3	14 ± 3	18 ± 3	0.26
	Schedule II	0 Gy	Control	69 ± 3	21 ± 3	11 ± 2	0.16
			NVP-BEZ235	82 ± 2	12 ± 1	6 ± 2	0.07
		8 Gy	Control	54 ± 2	20 ± 2	27 ± 2	0.50
			NVP-BEZ235	55 ± 2	8 ± 2	37 ± 1	0.67
GaMG	Schedule I	0 Gy	Control	87 ± 4	5 ± 3	8 ± 2	0.09
			NVP-BEZ235	81 ± 4	10 ± 3	10 ± 2	0.12
		8 Gy	Control	66 ± 8	12 ± 6	22 ± 4	0.33
			NVP-BEZ235	65 ± 4	18 ± 4	17 ± 3	0.26
	Schedule II	0 Gy	Control	77 ± 8	12 ± 5	11 ± 3	0.14
			NVP-BEZ235	80 ± 5	10 ± 3	10 ± 3	0.13
		8 Gy	Control	55 ± 6	21 ± 6	25 ± 6	0.45
			NVP-BEZ235	40 ± 7	11 ± 4	49 ± 9	1.23
U373	Schedule I	0 Gy	Control	82 ± 4	8 ± 2	10 ± 3	0.12
			NVP-BEZ235	79 ± 4	9 ± 2	12 ± 2	0.15
		8 Gy	Control	62 ± 8	16 ± 4	22 ± 5	0.35
			NVP-BEZ235	64 ± 5	15 ± 4	21 ± 3	0.33
	Schedule II	0 Gy	Control	70 ± 3	14 ± 2	16 ± 2	0.23
			NVP-BEZ235	68 ± 2	16 ± 2	16 ± 1	0.24
		8 Gy	Control	38 ± 5	22 ± 3	40 ± 4	1.05
			NVP-BEZ235	12 ± 5	14 ± 2	74 ± 6	6.17

Forty-eight hours after IR, cells were fixed, permeabilized, stained with PI, and analyzed for DNA content by flow cytometry. Data are presented as means (±SD) from at least four independent experiments. For detailed description, see legend to Figure 6.