

Expo, 82-6 hTert

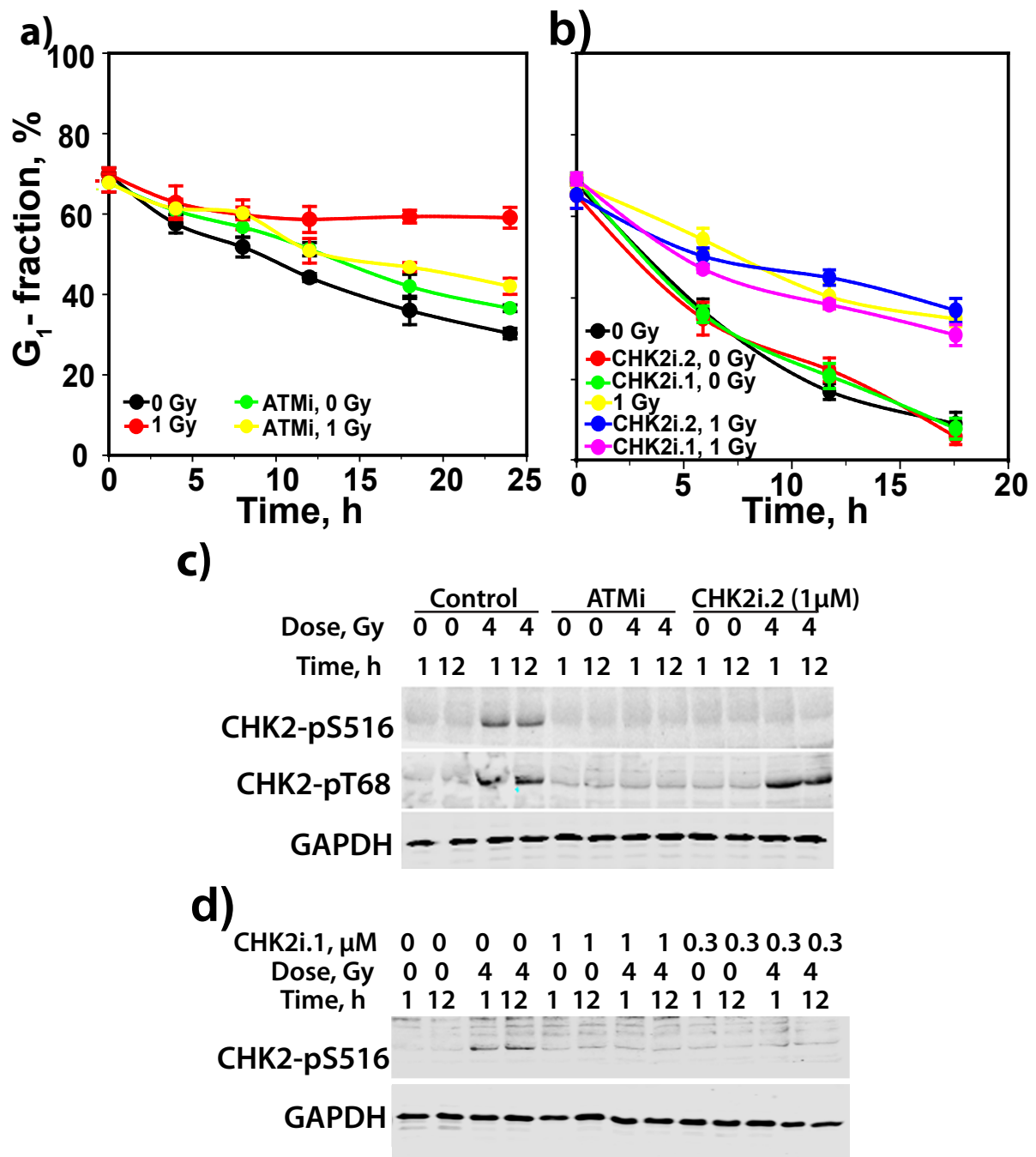


Figure S1, Li et al.

Figure S1. Demonstration of IR-induced G1-checkpoint in G1-irradiated 82-6hTert cells. a) As in Figure 1b for cells exposed to 0 or 1 Gy of X-rays in the presence or absence of ATMi as indicated. b) As in a for cells exposed to 0 or 1 Gy and the indicated inhibitors of CHK1 and CHK2. c) Effects of ATMi or CHK2i.2 on the phosphorylation of CHK2 in exponentially growing 82-6 hTert cells. d) Effects of CHK2i.1 on the phosphorylation of CHK2-S516 in exponentially growing 82-6 hTert cells. In a) and b), plotted is the mean and SE from three independent experiments. See also Table S6 for more information. In c) and d), experiments are reproduced 3 times, and a representative one is shown.

# Expo, 82-6 htert

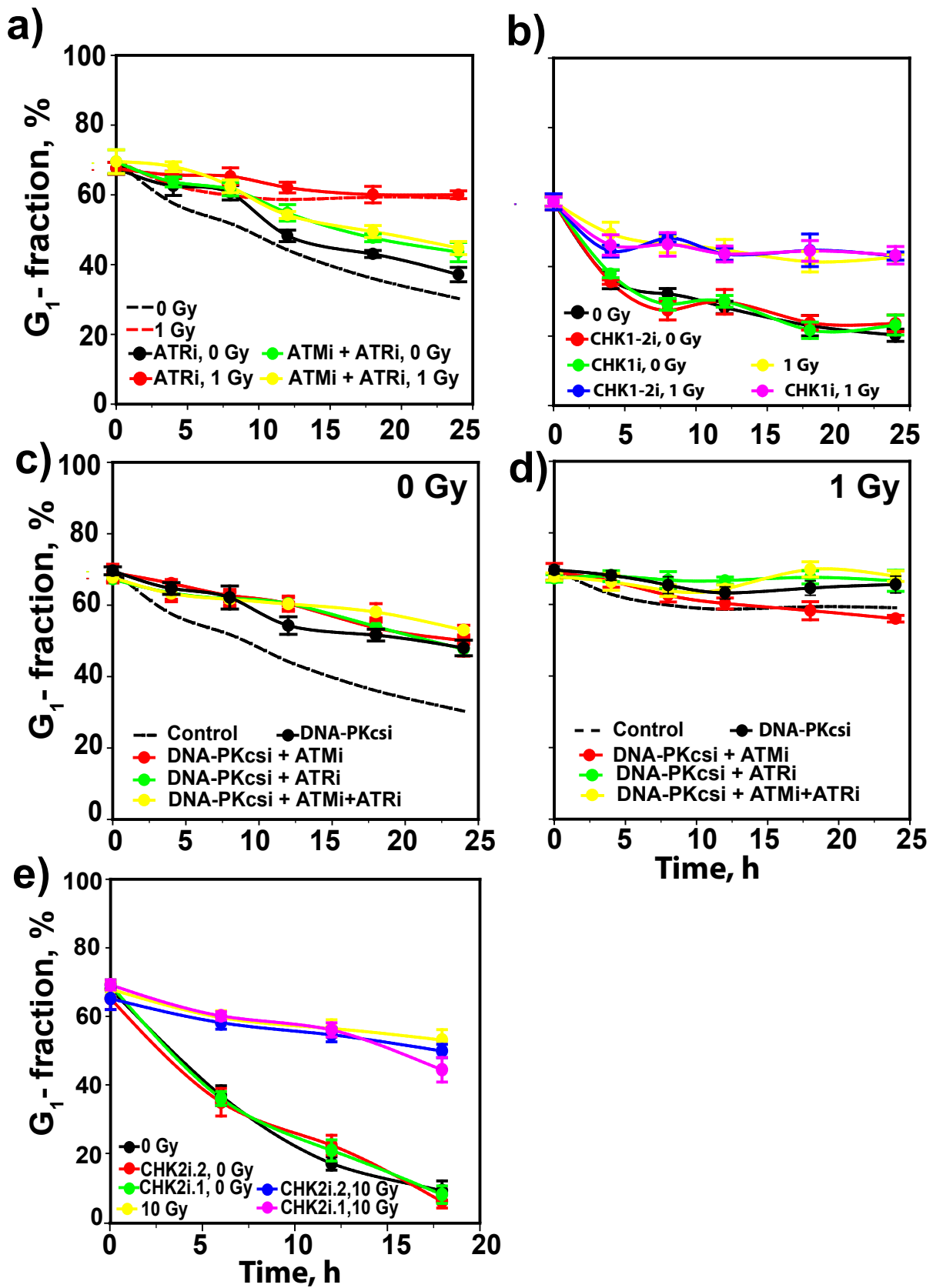
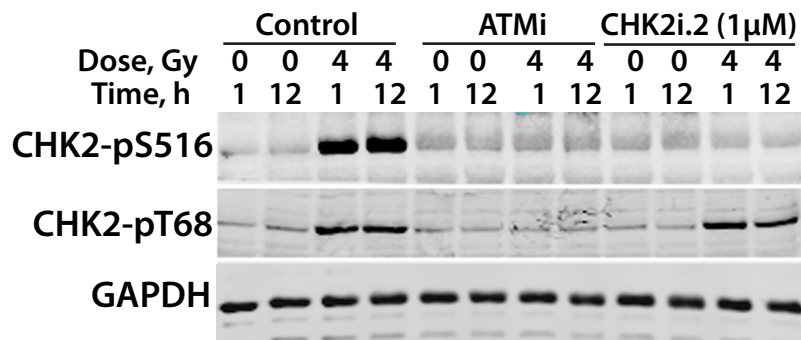


Figure S2, Li et al.

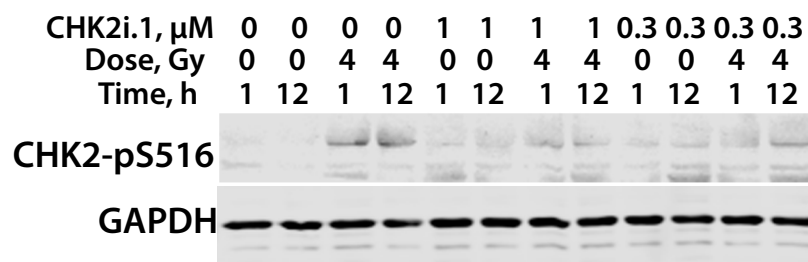
Figure S2. Effects of combinations of ATM, ATR, CHK1, CHK2 and DNA-PKcs inhibitors on low-IR-dose-induced G<sub>1</sub> checkpoint in G<sub>1</sub> irradiated 82-6 hTert cells. a) As in Figure 1b for cells exposed to 0 or 1 Gy and the indicated combinations of ATMi and ATRi. b) As in a for cells exposed to CHK1 and/or CHK2 inhibitors as indicated. c) as in a for unirradiated cells exposed to ATMi, ATRi and DNA-PKcsi as indicated. d) As in c for 1Gy irradiated cells exposed to ATMi, ATRi and DNA-PKcsi as indicated. e) As in a for cells exposed to 0 or 10 Gy and the indicated inhibitors of CHK2. Broken lines show results from Figure S1 without data point to avoid congestion. Plotted is the mean and SE from three independent experiments. See also Table S7 for more information.

## G<sub>0</sub>, release, 82-6 hTert

**a)**



**b)**



**Figure S3, Li et al.**

Figure S3. As in Figure S1c and S1d for G<sub>0</sub> cells released into the cell cycle by transfer to fresh growth medium just before irradiation and application of the indicated inhibitors. Experiments are reproduced 3 times, and a representative one is shown.

$G_0$ , 82-6 hTert

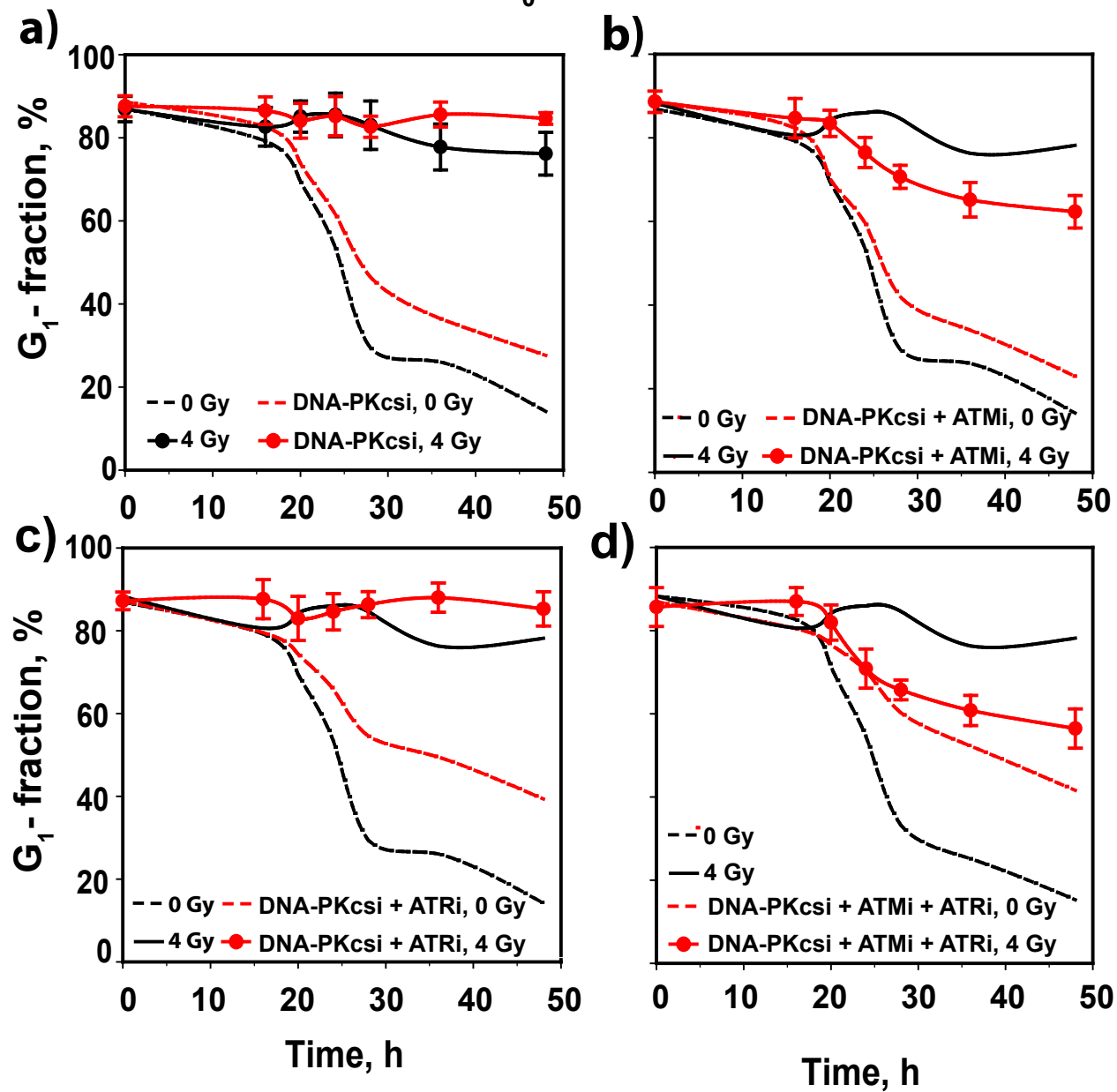
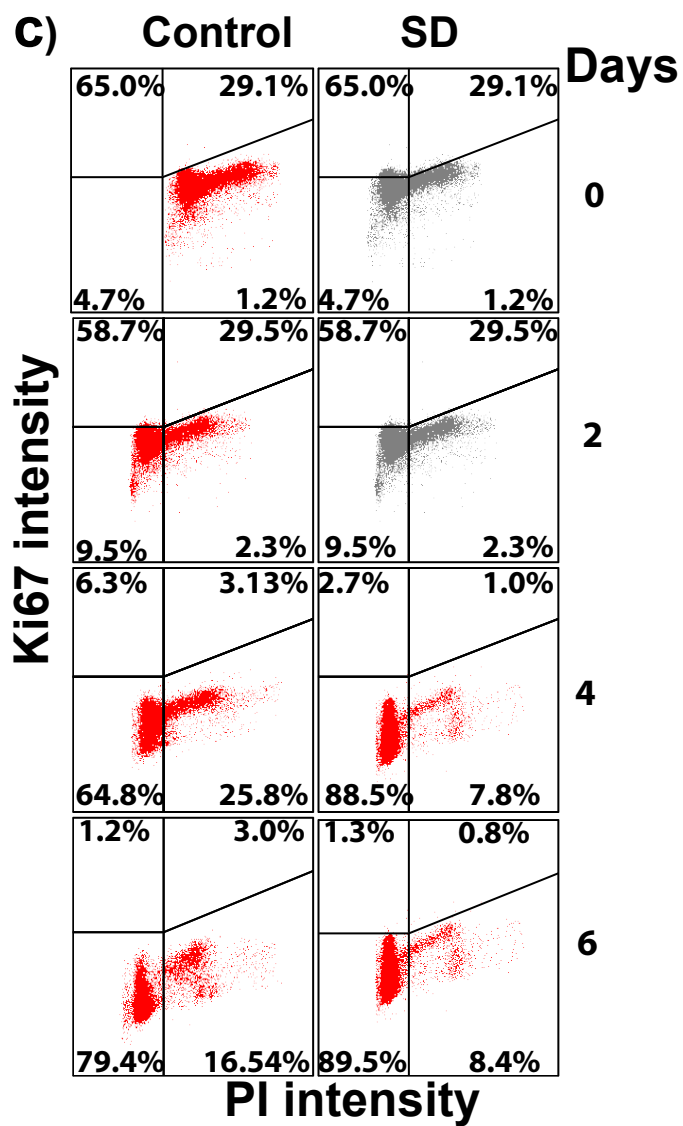
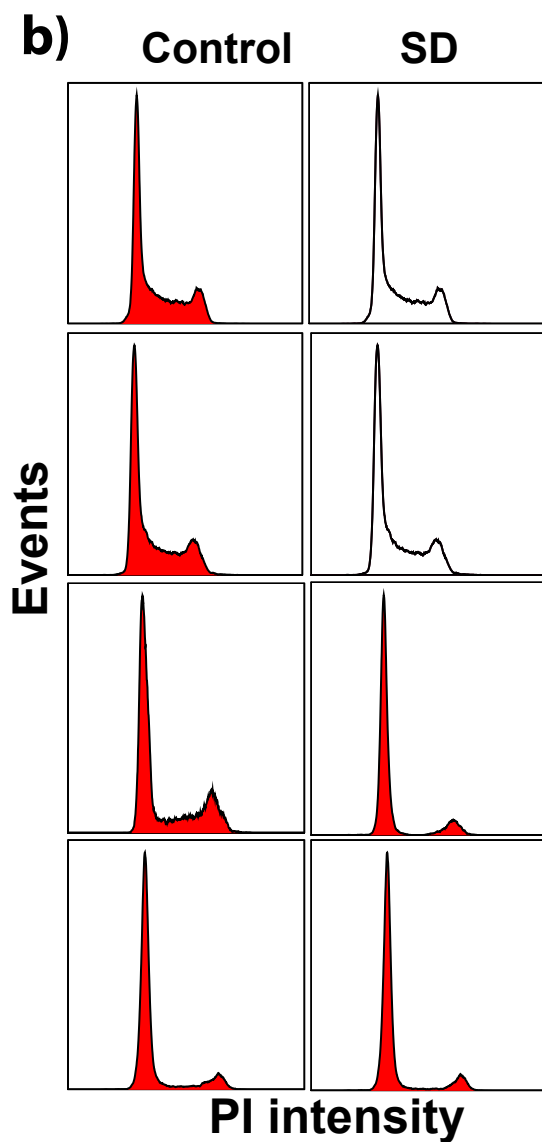
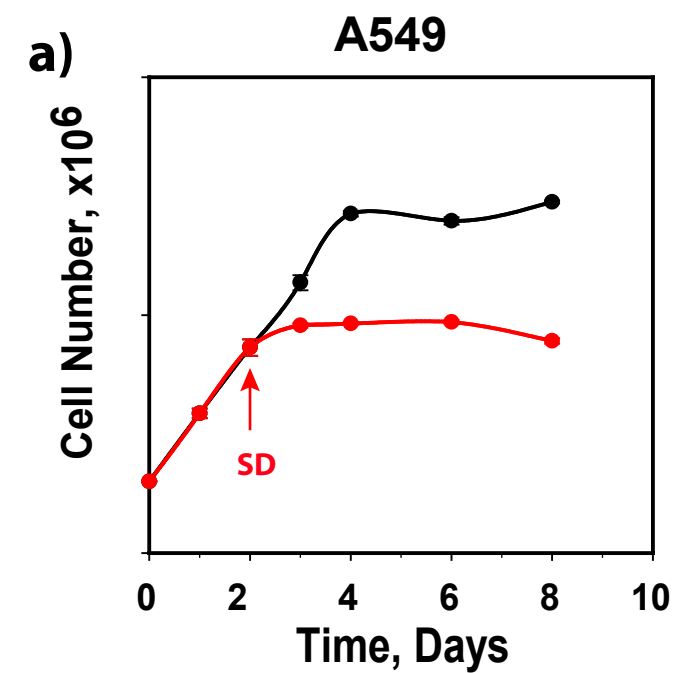


Figure S4 Li et al.

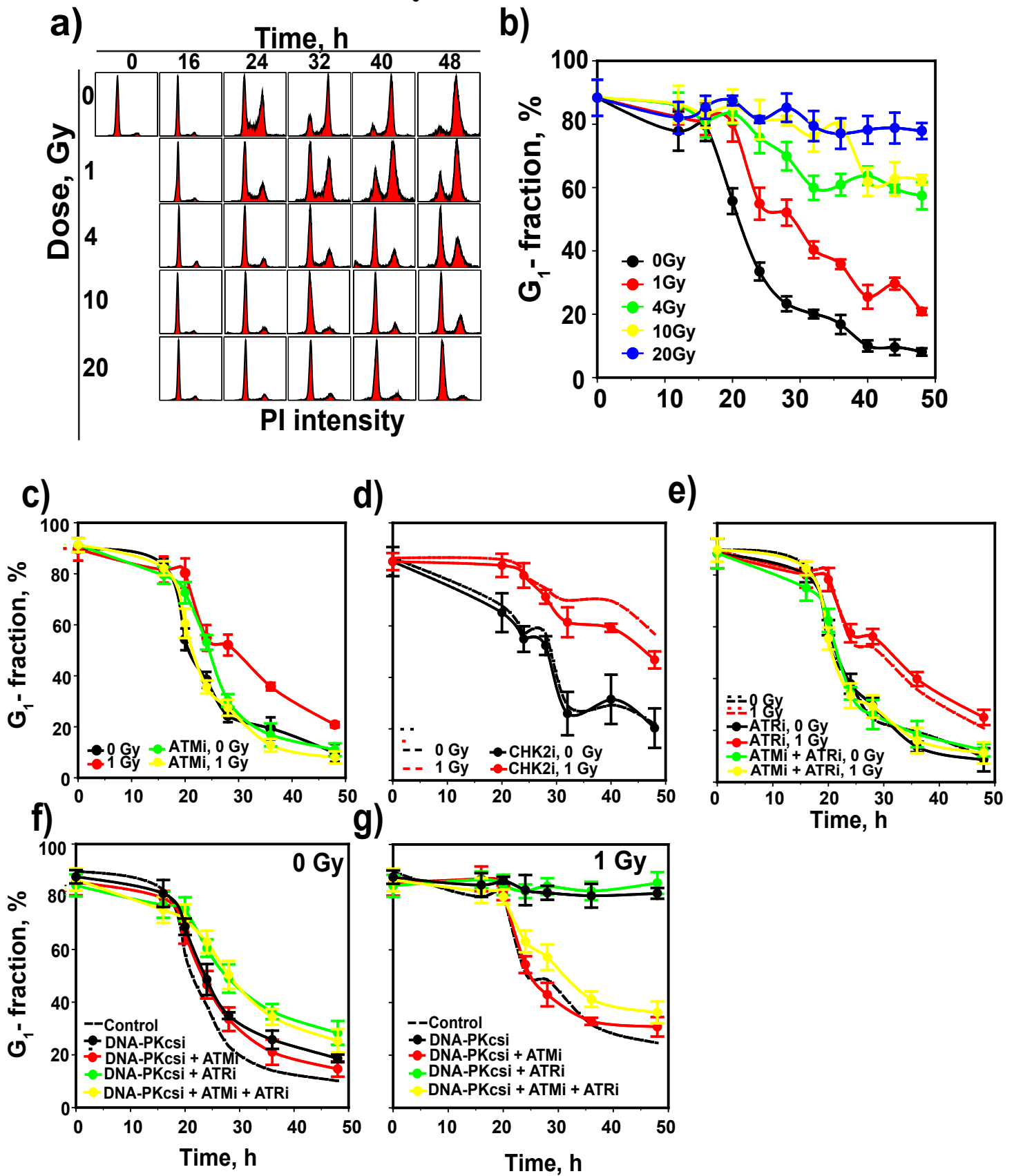
Figure S4. a) As in 1b for G<sub>0</sub> cells SD 82-6 hTert cells exposed to 0 or 4 Gy and treated with DNA-PKcsi as indicated. b) As in a for cells treated with combinations of ATMi and DNA-PKcsi as indicated. c) As in b for cells treated with combinations of ATRi and DNA-PKcsi inhibitors as indicated. d) As in c for cells treated with combined ATMi, ATRi and DNA-PKcsi. Lines of both broken and solid show results from Figure 4, or S4a without data point to avoid congestion. Plotted is the mean and SE from three independent experiments. See also Table S8 for more information



Li et al, Figure S5

Figure S5. Validation of A549 cell cultures for the analysis of IR-induced G1-checkpoint in cells irradiated in G<sub>0</sub>. a) Proliferation of A549 cells under normal growth conditions, as well as following SD after two days. Plotted is the mean and SE from three independent experiments. b) Cell cycle distribution at the indicated times for cells growing as in a. c) Characterization of cell cultures in a for Ki67 signal. In b) and c), experiments are reproduced 3 times and a representative one is shown.

$G_0$ , A549



Li et al, Figure S6

Figure S6. IR-induced G1 checkpoint in G0 irradiated A549 cells. a) Representative flow cytometer histograms after irradiation and transfer to fresh growth medium obtained from 3 independent experiments. b) As in Figure 3b for A549 cells exposed to different doses of IR. c) As in b for cells exposed to 0 or 1 Gy and treated with ATMi. d) As in c for cells treated with CHK2i. e) As in c for cells treated with combinations of ATMi and ATRi. f) As in c for unirradiated cells treated with the indicated combinations of DNA-PKcsi, ATMi and ATRi. g) As in f for cells exposed to 1 Gy. Broken lines show results from c without data point to avoid congestion. Plotted is the mean and SE from three independent experiments. See also Table S9 for more information.

$G_0$ , A549

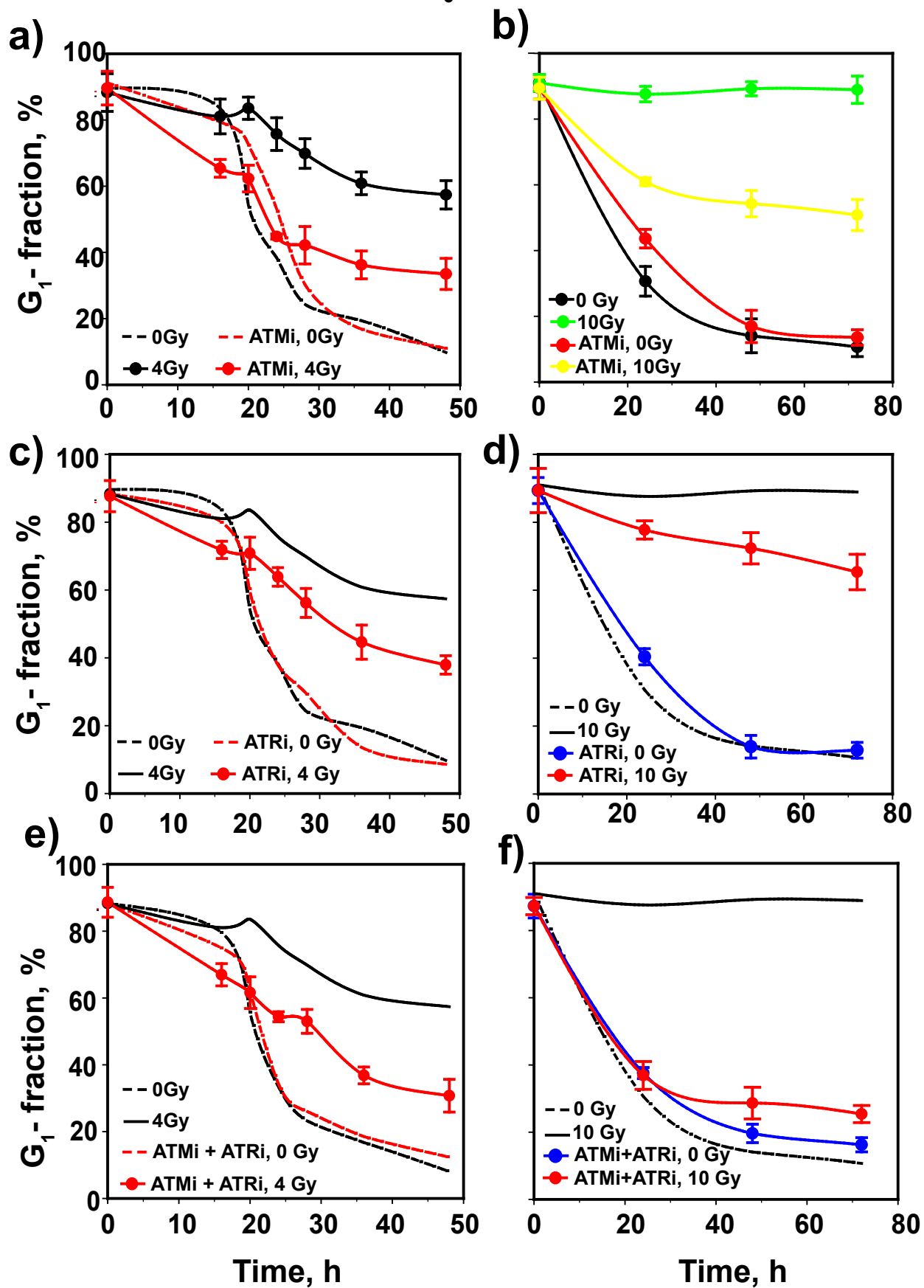
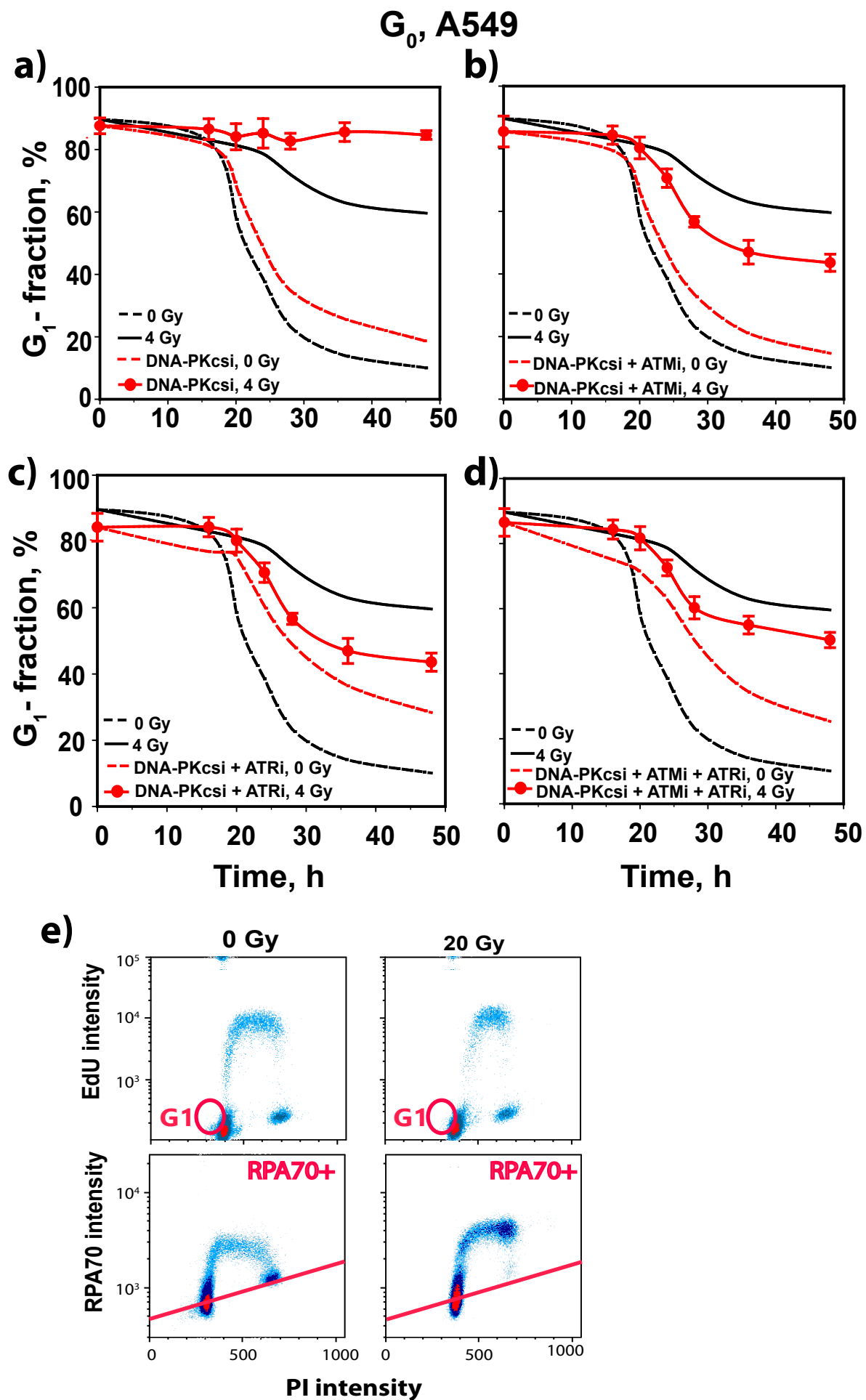


Figure S7 Li et al.

Figure S7. As in Figure 5 for A549 cells. Lines of both broken and solid show results from Figure S6 or S7b without data point to avoid congestion. See also Table S10 for more information.



**Figure S8, Li et al.**

Figure S8. a)-d) As in Figure S4 for A549 cells. Lines of both broken and solid show results from Figure S6 or S7 without data point to avoid congestion. Plotted is the mean and SE from three independent experiments. See also Table S11 for more information. e) Example of the approach used to quantitate DNA-end-resection at DSBs by measuring RPA70 intensity in G1-phase of cells. The upper panel shows the gating of G1-phase cells using PI and EdU signal. The lower panel shows the gating used for the detection of RPA70 signal.

**Table S1. Statistical analyses of Figure 1**

<b>Fig 1b</b>	<b>0Gy Vs 1Gy</b>	<b>1Gy Vs ATMi, 1Gy</b>	<b>1Gy Vs ATRi 1Gy</b>
<b>16h after IR</b>	<b>**</b>	<b>**</b>	<b>N. S</b>
<b>24h after IR</b>	<b>**</b>	<b>**</b>	<b>N. S</b>

<b>Fig1c</b>	<b>0Gy Vs 10Gy</b>	<b>ATMi, 0Gy Vs ATMi, 10Gy</b>	<b>10Gy Vs ATMi, 10Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>

<b>Fig 1d</b>	<b>0Gy Vs 10Gy</b>	<b>10Gy Vs ATRi, 10Gy</b>	<b>10Gy Vs CHK1i, 10Gy</b>	<b>10Gy Vs CHK1-2i, 10Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>*</b>	<b>**</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>N. S</b>	<b>*</b>	<b>*</b>

<b>Fig 1e</b>	<b>ATMi+ATRi, 0Gy Vs ATMi+ATRi, 10Gy</b>	<b>ATMi+CHK1i, 0Gy Vs ATMi+CHK1i,10Gy</b>
<b>36h after IR</b>	<b>N. S</b>	<b>N. S</b>
<b>48h after IR</b>	<b>N. S</b>	<b>N. S</b>

<b>Fig 1f</b>	<b>0Gy Vs DNA-PKcs, 0Gy</b>	<b>DNA-PKcsi, 0Gy Vs DNA-PKcs, 10Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>**</b>

**Table S2. Statistical analyses of Figure 3**

<b>Fig 3b</b>	<b>0Gy Vs 1Gy</b>	<b>0Gy Vs 4 Gy</b>	<b>0Gy Vs 10Gy</b>	<b>0Gy Vs 20Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>	<b>**</b>
<b>40h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>	<b>**</b>

**Table S3. Statistical analyses of Figure 4**

Fig 4a	0Gy Vs 1Gy	ATMi, 0Gy Vs ATMi, 1Gy	1 Gy Vs ATMi, 1Gy
36h after IR	**	N. S	**
48h after IR	**	N. S	**
Fig 4b	1Gy Vs CHK2i2, 1Gy	1 Gy Vs CHK2i1, 1Gy	
36h after IR	N. S	N. S	
48h after IR	N. S	N. S	
Fig 4c	1Gy Vs ATRi, 1Gy	1Gy Vs CHK1-2i, 1Gy	1Gy Vs CHK1i, 1Gy
36h after IR	**	N. S	N. S
48h after IR	**	N. S	N. S
Fig 4f	0Gy Vs 10Gy	10Gy Vs CHK2i2, 10Gy	10 Gy Vs CHK2i1, 10Gy
36h after IR	**	N. S	N. S
48h after IR	**	N. S	N. S

Table S4. Statistical analyses of Figure 5

Fig 5a	0Gy Vs 4Gy	ATMi, 0Gy Vs ATMi, 4Gy	4 Gy Vs ATMi, 4Gy
36h after IR	**	*	**
48h after IR	**	**	**

Fig 5b	0Gy Vs 10Gy	ATMi, 0Gy Vs ATMi, 10Gy	10 Gy Vs ATMi, 10Gy
36h after IR	**	**	**
48h after IR	**	**	**

Fig 5c	ATRi, 0Gy Vs ATRi, 4Gy	4 Gy Vs ATRi, 4Gy
36h after IR	**	**
48h after IR	**	**

Fig 5d	0Gy Vs 10Gy	10Gy Vs ATRi, 10Gy	10Gy Vs CHK1i, 10Gy	10Gy Vs CHK1-2i, 10Gy
36h after IR	**	**	**	**
48h after IR	**	**	**	**

Fig 5e	ATMi+ATRi 0Gy Vs ATMi+ATRi, 4Gy	4 Gy Vs ATMi+ATRi, 4Gy
36h after IR	N. S	**
48h after IR	N. S	**

Fig 5f	ATMi+ATRi, 0Gy Vs ATMi+ATRi,10Gy	ATMi+CHK1i, 0Gy Vs ATMi+CHK1i,10Gy	10Gy Vs ATMi+ATRi, 10Gy	10Gy Vs ATMi+CHK1i,10Gy
36h after IR	N. S	N. S	**	**
48h after IR	N. S	N. S	**	**

**Table S5. Statistical analyses of Figure 6b**

	ATMi	ATRi	ATMi+ATRi	DNA-PKcsi	ATMi+DNA -PKcsi	ATRi+DNA- PKcsi	ATMi+ATRi+ DNA-PKcsi
<b>Un. tr</b>	*	**	**	N. S	**	*	**

**Table S6. Statistical analyses of Figure S1**

<b>Fig S1a</b>	<b>0Gy Vs 1Gy</b>	<b>ATMi, 0Gy Vs ATMi, 1Gy</b>	<b>1 Gy Vs ATMi, 1Gy</b>
<b>18h after IR</b>	<b>**</b>	<b>N. S</b>	<b>**</b>
<b>24h after IR</b>	<b>**</b>	<b>N. S</b>	<b>**</b>

<b>Fig S1b</b>	<b>0Gy Vs 1Gy</b>	<b>1Gy Vs CHK2i2, 1Gy</b>	<b>1 Gy Vs CHK2i1, 1Gy</b>
<b>8h after IR</b>	<b>**</b>	<b>N. S</b>	<b>N. S</b>
<b>16h after IR</b>	<b>**</b>	<b>N. S</b>	<b>N. S</b>

**Table S7. Statistical analyses of Figure S2**

<b>Fig S2a</b>	<b>ATRi, 0Gy Vs ATRi, 1Gy</b>	<b>ATMi+ATRi, 0Gy Vs ATMi+ATRi,1Gy</b>	<b>1Gy Vs ATRi, 1Gy</b>	<b>1Gy Vs ATMi+ATRi,1Gy</b>
<b>18h after IR</b>	<b>**</b>	<b>N. S</b>	<b>N. S</b>	<b>**</b>
<b>24h after IR</b>	<b>**</b>	<b>N. S</b>	<b>N. S</b>	<b>**</b>

<b>Fig S2b</b>	<b>0Gy Vs 1Gy</b>	<b>CHK1i, 0Gy Vs CHK1i,1Gy</b>	<b>CHK1-2i, 0Gy Vs CHK1-2i, 1Gy</b>
<b>18h after IR</b>	<b>**</b>	<b>N. S</b>	<b>N. S</b>
<b>24h after IR</b>	<b>**</b>	<b>N. S</b>	<b>N. S</b>

<b>Fig S2e</b>	<b>0Gy Vs 10Gy</b>	<b>CHK2i2, 0Gy Vs CHK2i2,10Gy</b>	<b>CHK2i1, 0Gy Vs CHK2i1, 10Gy</b>
<b>8h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>
<b>16h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>

Table S8. Statistical analyses of Figure S4

Fig S4a	0Gy Vs 4Gy	DNA-PKcsi, 0Gy Vs DNA-PKcsi, 4Gy	4Gy Vs DNA-PKcsi, 4Gy
36h after IR	**	**	*
48h after IR	**	**	*
Fig S4b	DNA-PKcsi+ATMi, 0Gy Vs DNA-PKcsi+ATMi, 4Gy	4Gy Vs DNA-PKcsi+ATMi, 4Gy	
36h after IR	**	**	
48h after IR	**	**	
Fig S4c	DNA-PKcsi+ATRi, 0Gy Vs DNA-PKcsi+ATRi, 4Gy	4Gy Vs DNA-PKcsi+ATRi, 4Gy	
36h after IR	**	*	
48h after IR	**	N. S	
Fig S4d	DNA-PKcsi+ATMi+ATRi, 0Gy Vs DNA-PKcsi+ATMi+ATRi, 4Gy	4Gy Vs DNA-PKcsi+ATMi+ATRi, 4Gy	
36h after IR	N. S	**	
48h after IR	*	**	

**Table S9. Statistical analyses of Figure S6**

<b>Fig S6b</b>	<b>0Gy Vs 1Gy</b>	<b>0Gy Vs 4Gy</b>	<b>0Gy Vs 10Gy</b>	<b>0Gy Vs 20Gy</b>
<b>40h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>	<b>**</b>
<b>44h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>	<b>**</b>

<b>Fig S6c</b>	<b>0Gy Vs 1Gy</b>	<b>ATMi, 0Gy Vs ATMi, 1Gy</b>	<b>1 Gy Vs ATMi, 1Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>N. S</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>N. S</b>	<b>**</b>

<b>Fig S6d</b>	<b>0Gy Vs 1Gy</b>	<b>CHK2i, 0Gy Vs CHK2i, 1Gy</b>	<b>1 Gy Vs CHK2i, 1Gy</b>
<b>40h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>**</b>	<b>*</b>

<b>Fig S6e</b>	<b>ATRi, 0Gy Vs ATRi, 1Gy</b>	<b>ATMi+ATRi, 0Gy Vs ATMi+ATR, 1Gy</b>	<b>1 Gy Vs ATRi, 1Gy</b>	<b>1 Gy Vs ATMi+ATRi, 1Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>N. S</b>	<b>N. S</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>N. S</b>	<b>N. S</b>	<b>**</b>

**Table S10. Statistical analyses of Figure S7**

<b>Fig S7a</b>	<b>0Gy Vs 4Gy</b>	<b>ATMi, 0Gy Vs ATMi, 4Gy</b>	<b>4 Gy Vs ATMi, 4Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>N. S</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>N. S</b>	<b>**</b>

<b>Fig S7b</b>	<b>0Gy Vs 10Gy</b>	<b>ATMi, 0Gy Vs ATMi, 10Gy</b>	<b>10 Gy Vs ATMi, 10Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>**</b>	<b>**</b>

<b>Fig S7c</b>	<b>ATRi, 0Gy Vs ATRi, 4Gy</b>	<b>4Gy Vs ATRi, 4Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>*</b>
<b>48h after IR</b>	<b>**</b>	<b>**</b>

<b>Fig S7d</b>	<b>ATRi, 0Gy Vs ATRi, 10Gy</b>	<b>10 Gy Vs ATRi, 10Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>*</b>
<b>48h after IR</b>	<b>**</b>	<b>*</b>

<b>Fig S7e</b>	<b>ATMi+ATRi, 0Gy Vs ATMi+ATRi, 4Gy</b>	<b>4Gy Vs ATMi+ATRi, 4Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>**</b>

<b>Fig S7f</b>	<b>ATMi+ATRi, 0Gy Vs ATMi+ATRi, 10Gy</b>	<b>10Gy Vs ATMi+ATRi, 10Gy</b>
<b>36h after IR</b>	<b>N. S</b>	<b>**</b>
<b>48h after IR</b>	<b>N. S</b>	<b>**</b>

**Table S11. Statistical analyses of Figure S8**

<b>Fig S8a</b>	<b>DNA-Pkcsi, 0Gy Vs DNA-PKcsi, 4Gy</b>	<b>4Gy Vs DNA-PKcsi, 4Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>**</b>
<b>48h after IR</b>	<b>**</b>	<b>**</b>
<b>Fig S8b</b>	<b>DNA-PKcsi+ATMi, 0Gy Vs DNA-PKcsi+ATMi, 4Gy</b>	<b>4Gy Vs DNA-PKcsi+ATMi, 4Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>*</b>
<b>48h after IR</b>	<b>**</b>	<b>*</b>
<b>Fig S8c</b>	<b>DNA-PKcsi+ATRi, 0Gy Vs DNA-PKcsi+ATRi, 4Gy</b>	<b>4Gy Vs DNA-PKcsi+ATRi, 4Gy</b>
<b>36h after IR</b>	<b>N. S</b>	<b>*</b>
<b>48h after IR</b>	<b>*</b>	<b>**</b>
<b>Fig S8d</b>	<b>DNA-PKcsi+ATMi+ATRi, 0Gy Vs DNA-PKcsi+ATMi+ATRi, 4Gy</b>	<b>4Gy Vs DNA-PKcsi+ATMi+ATRi, 4Gy</b>
<b>36h after IR</b>	<b>**</b>	<b>N. S</b>
<b>48h after IR</b>	<b>**</b>	<b>*</b>