



Supplemental Material to:

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**Dissecting cellular responses to irradiation via targeted
disruptions of the ATM-CHK1-pp2a circuit**

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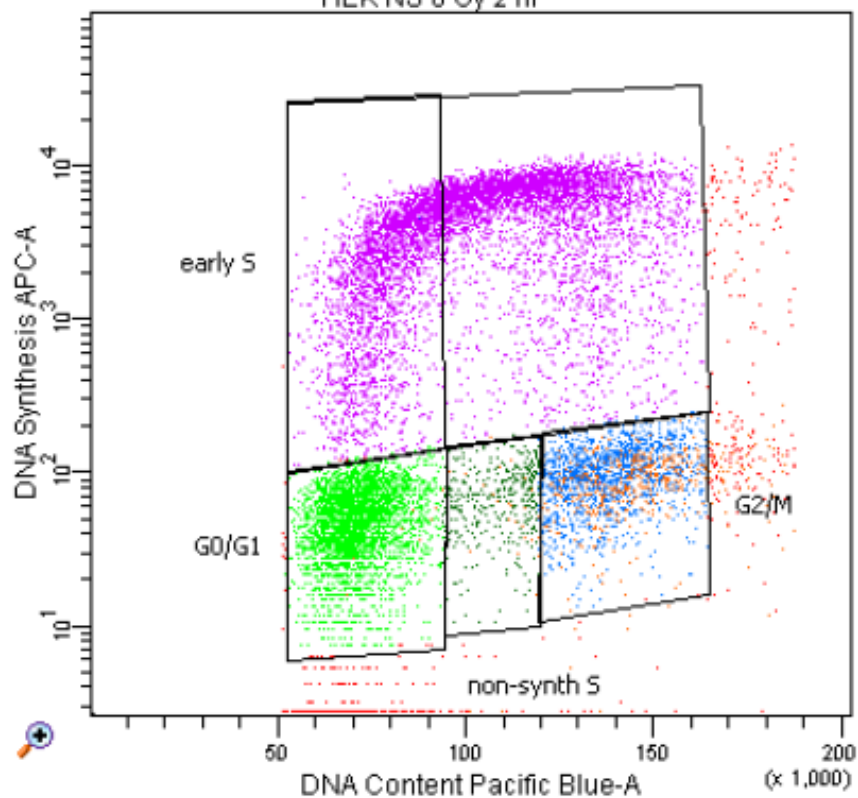
Supplementary data for Figure 3.

Flow cytometry profiles for HEK-NS and HEK-CHK1A cell lines. G2 and mitotic time course.

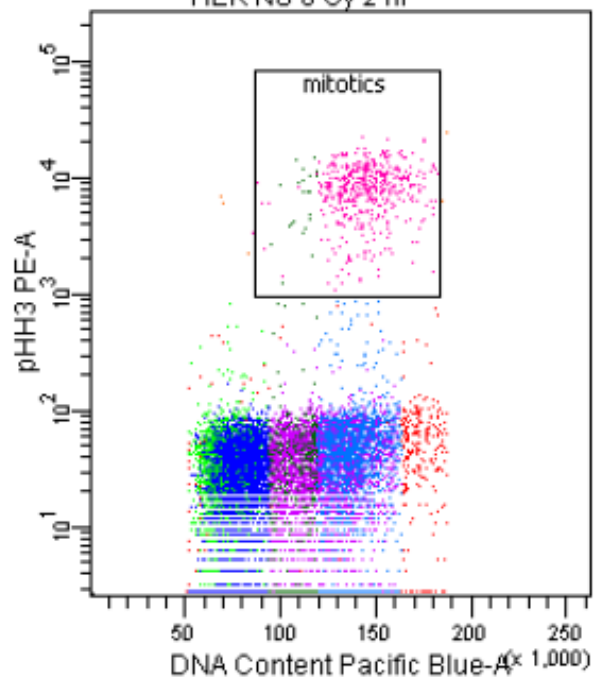
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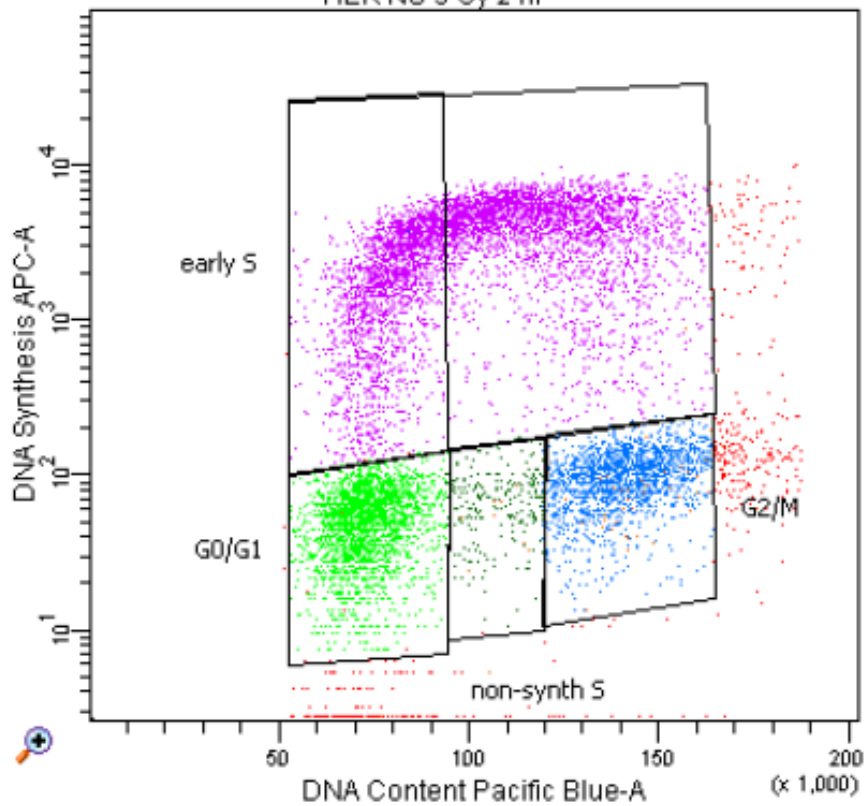
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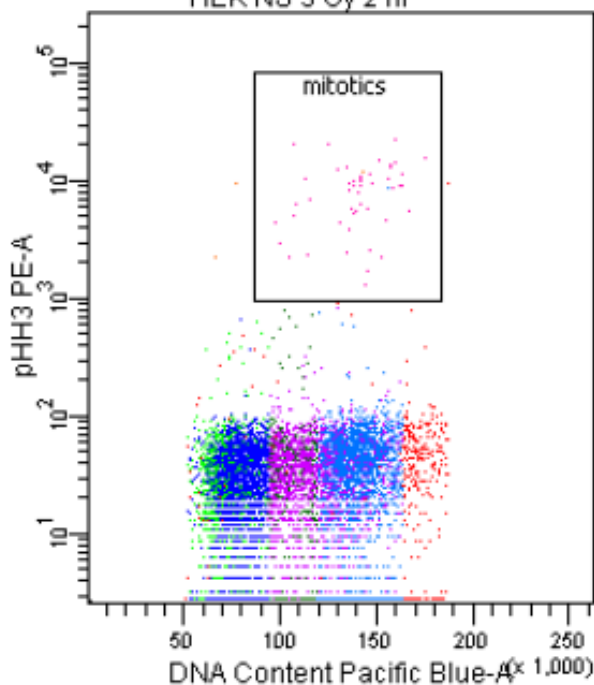
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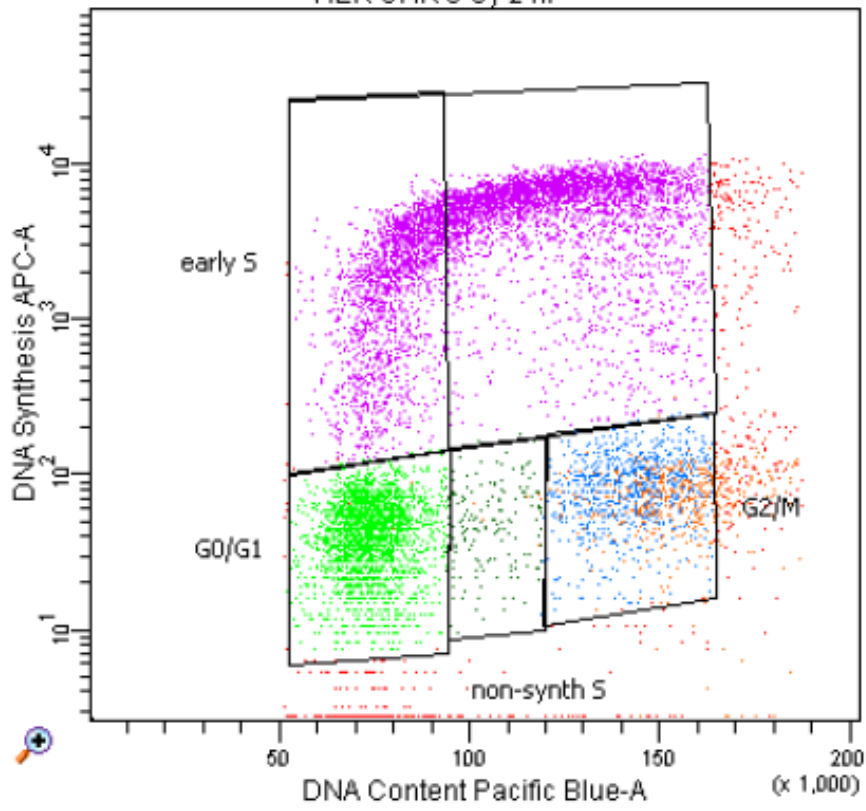
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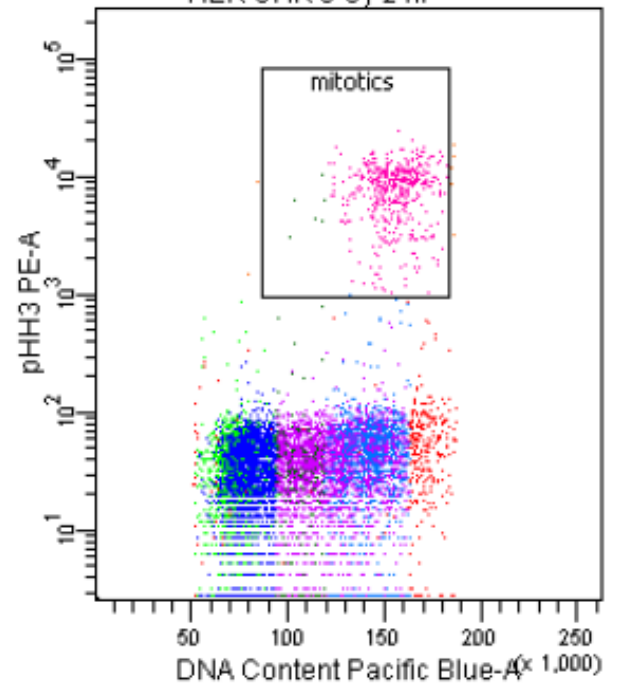
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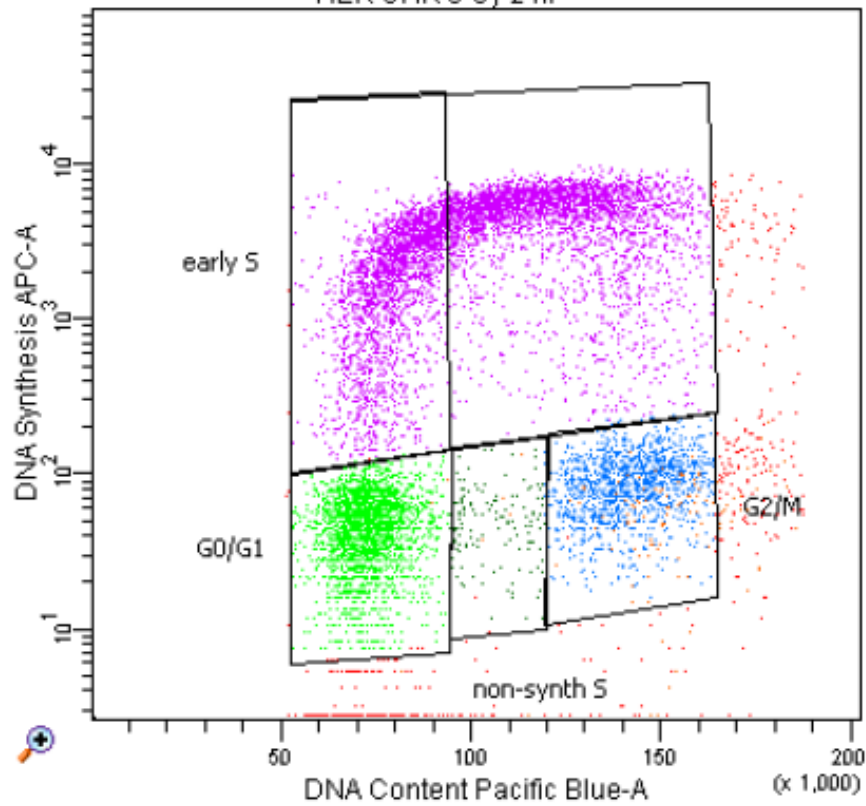
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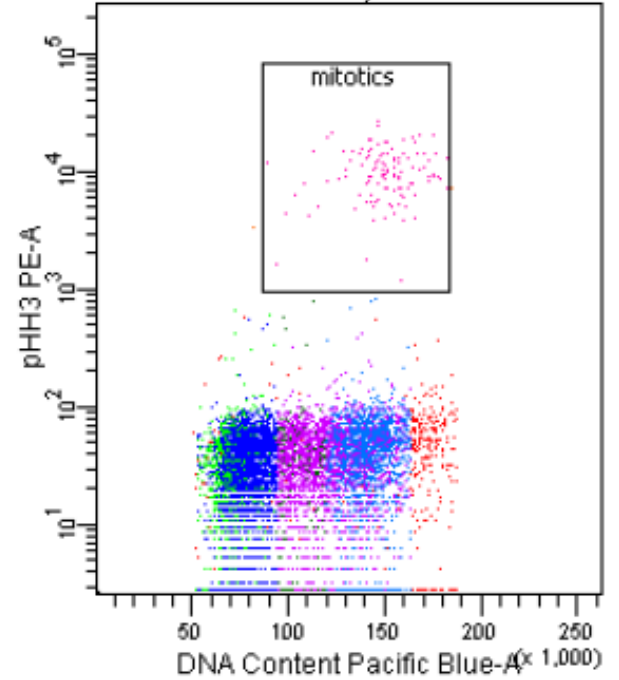
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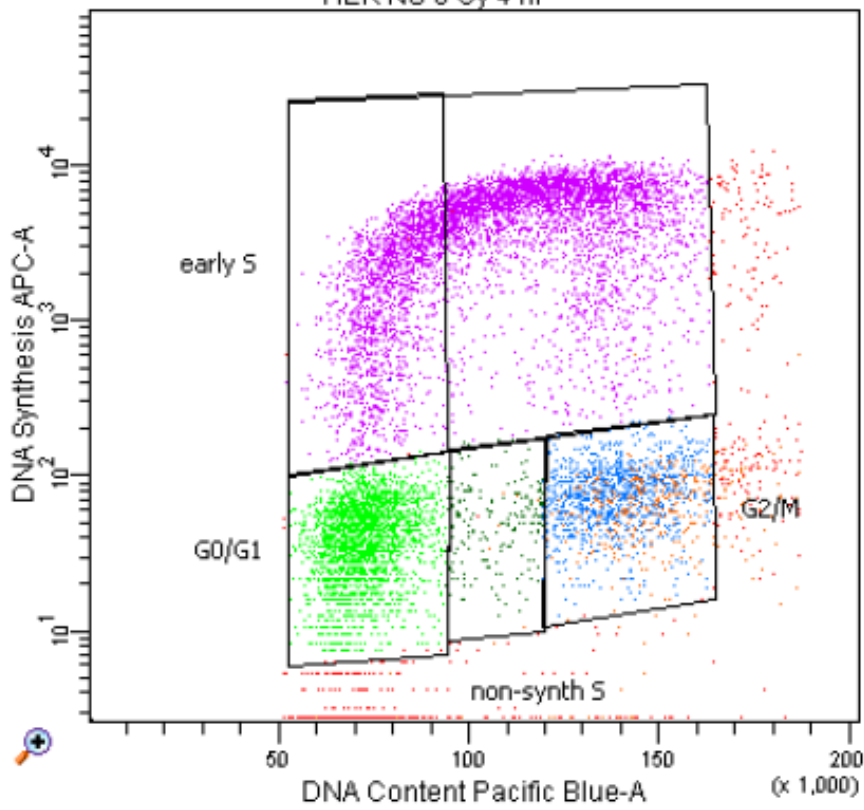
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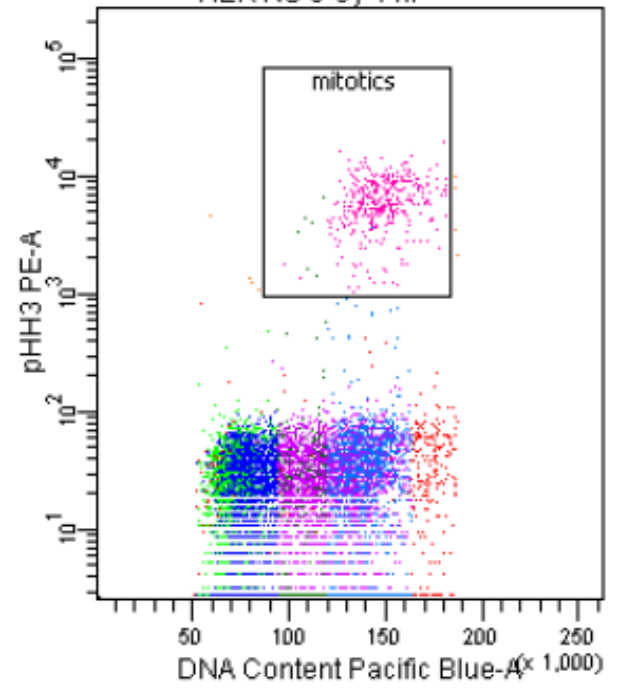
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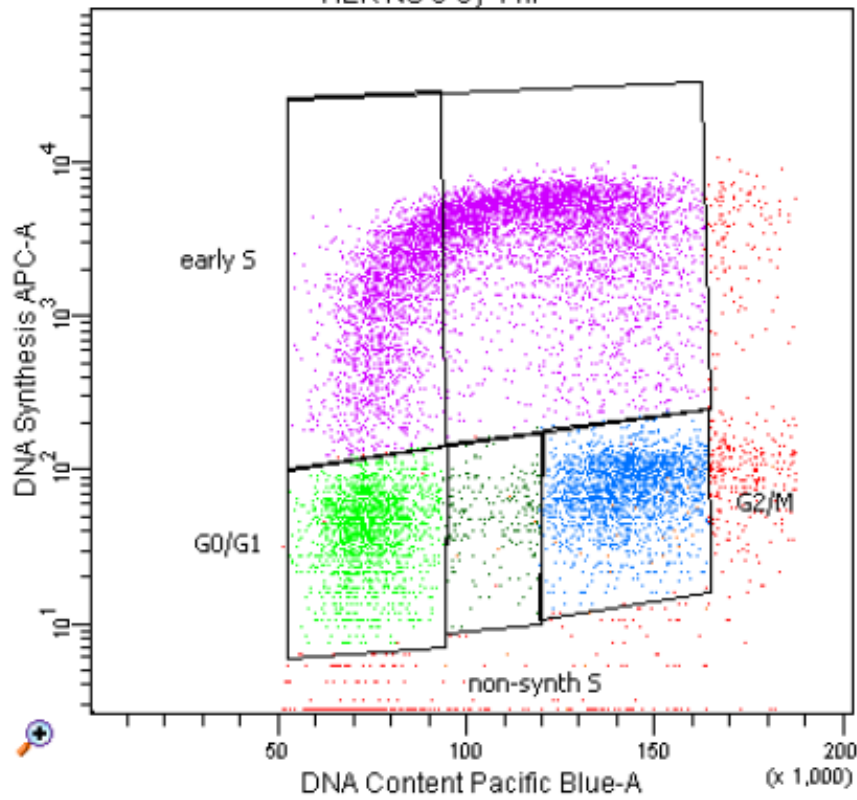
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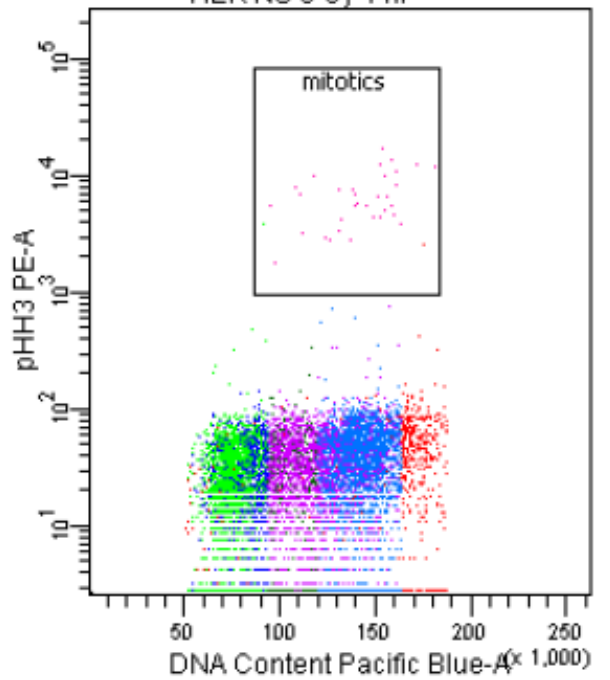
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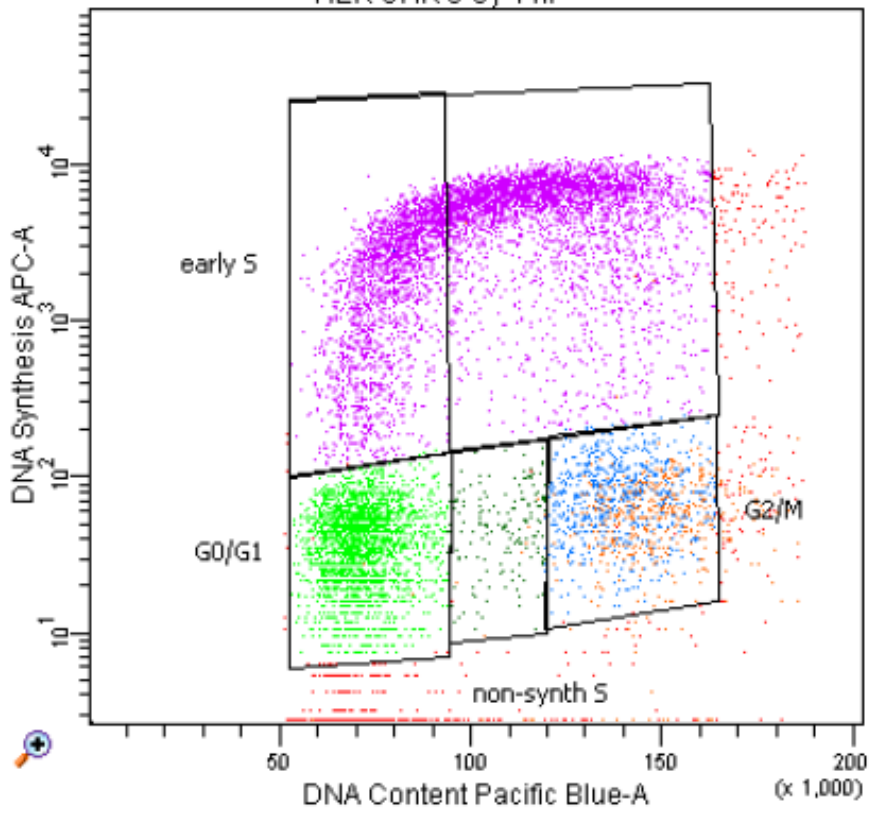
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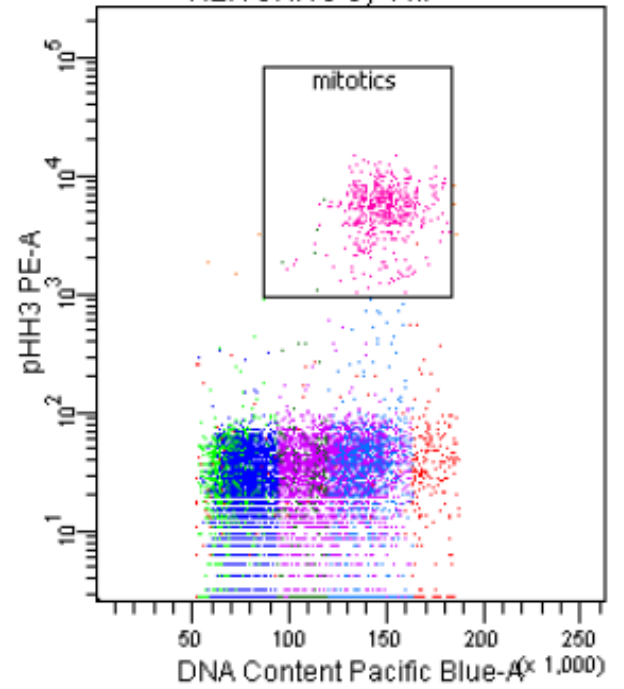
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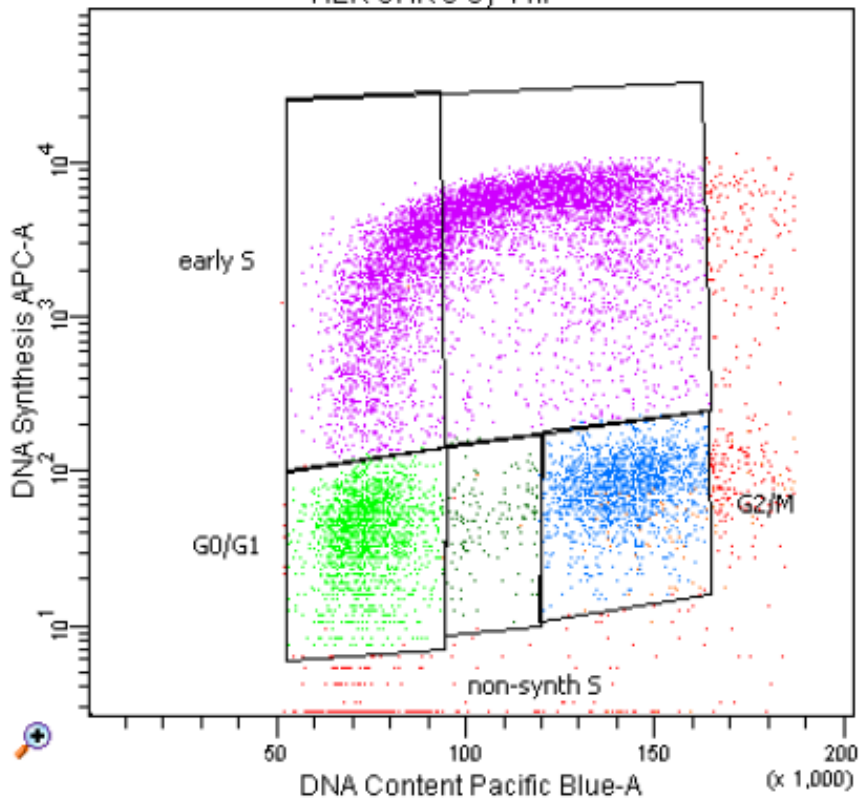
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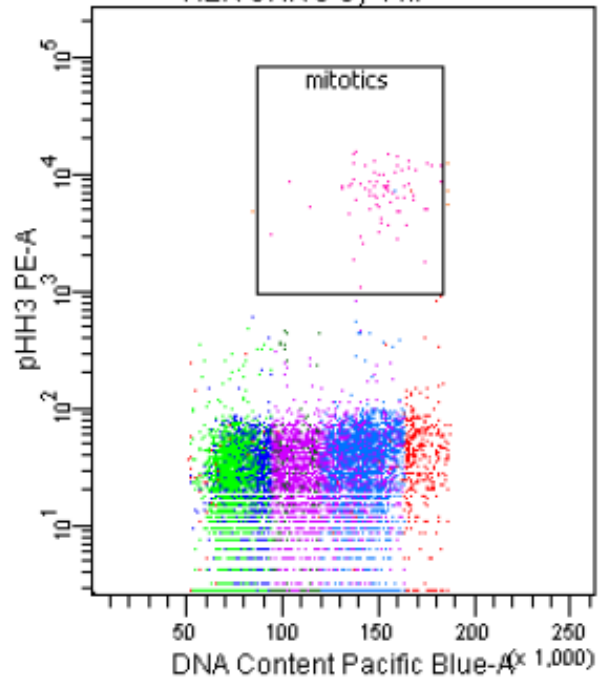
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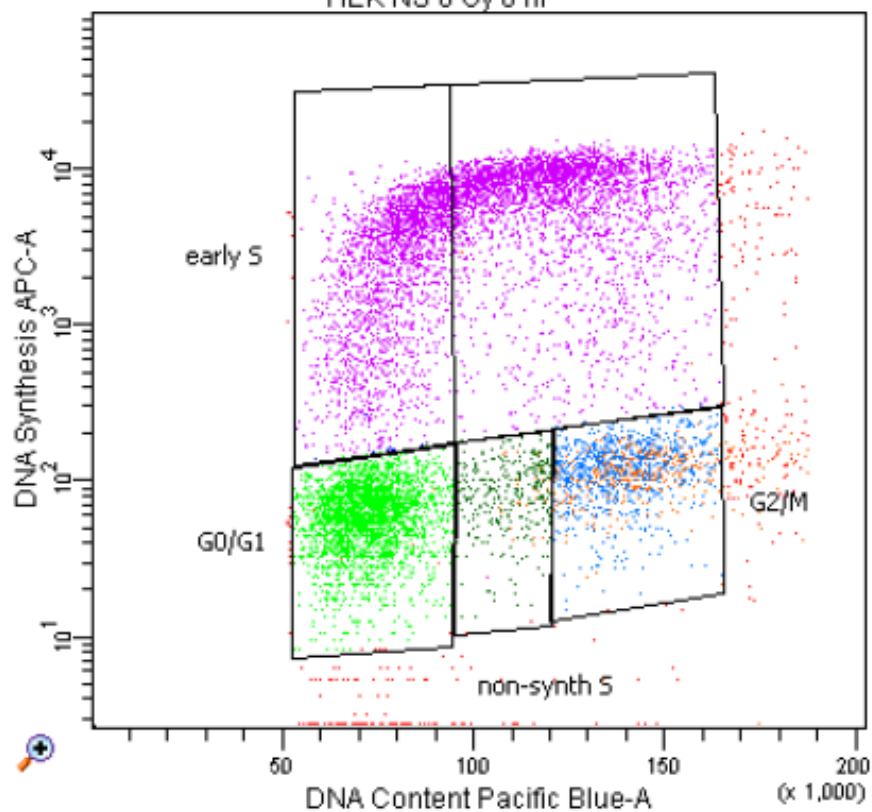
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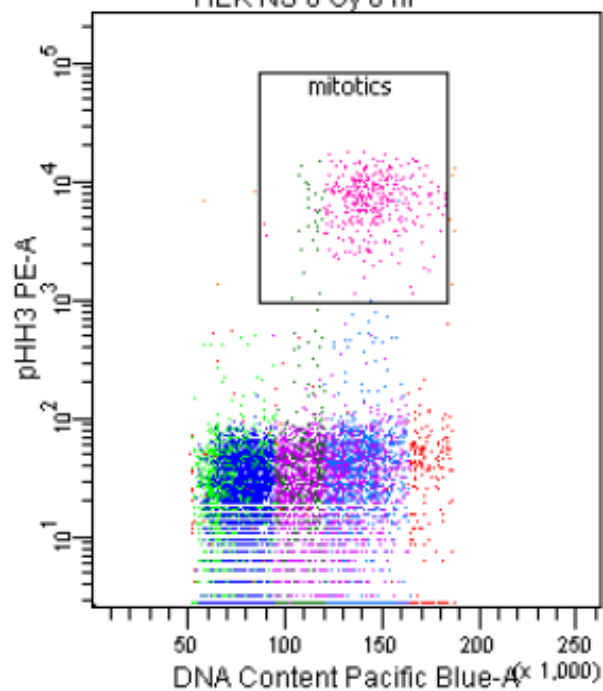
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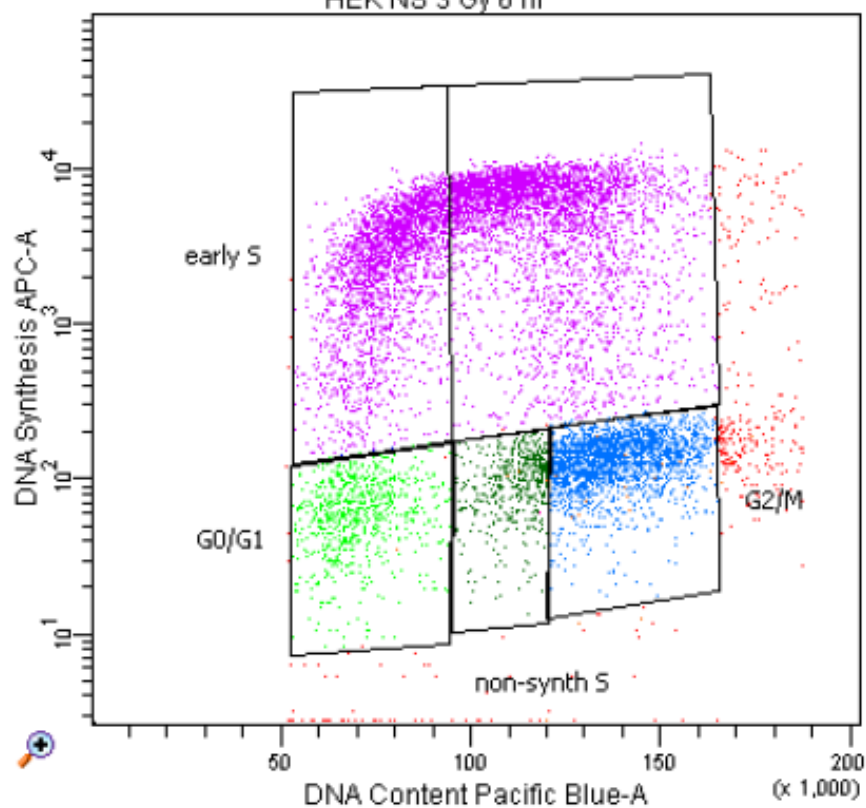
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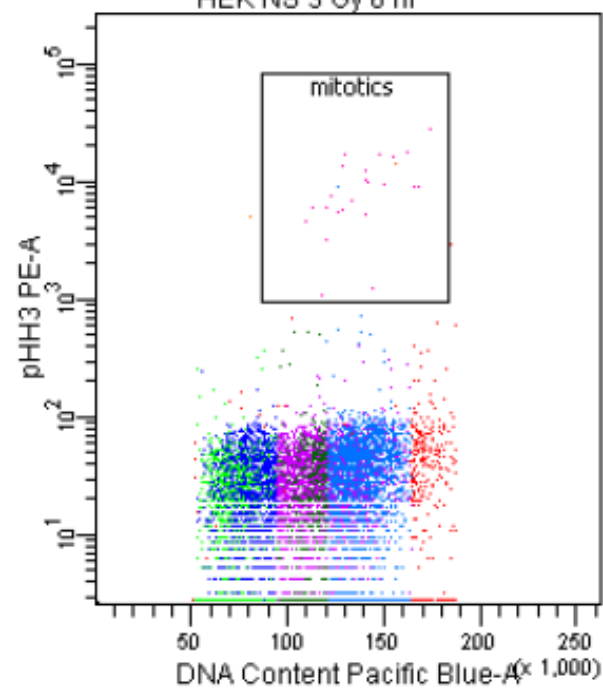
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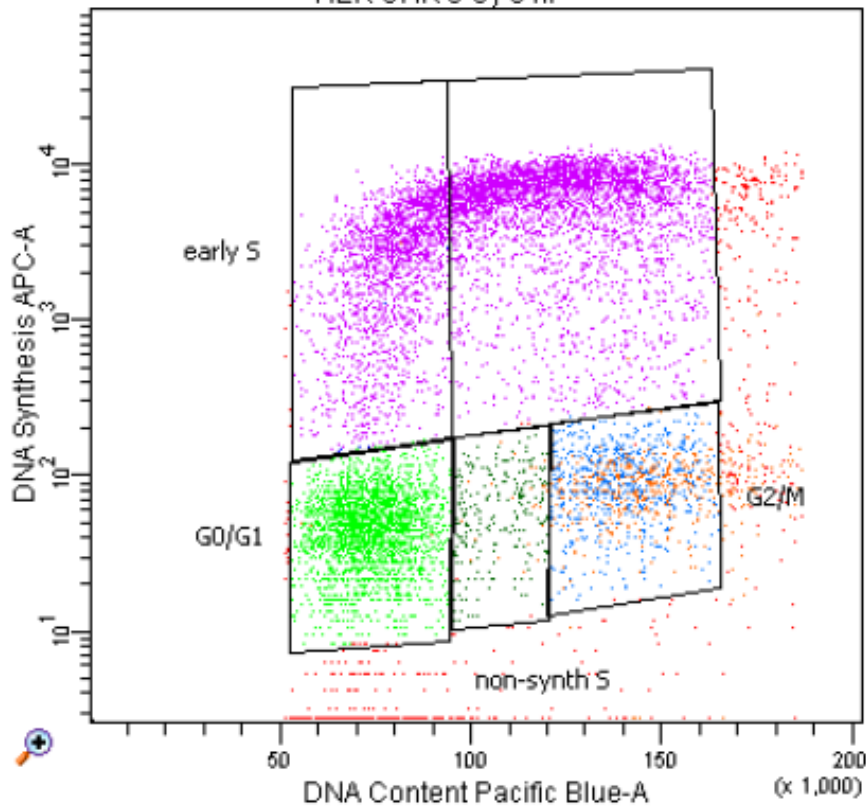
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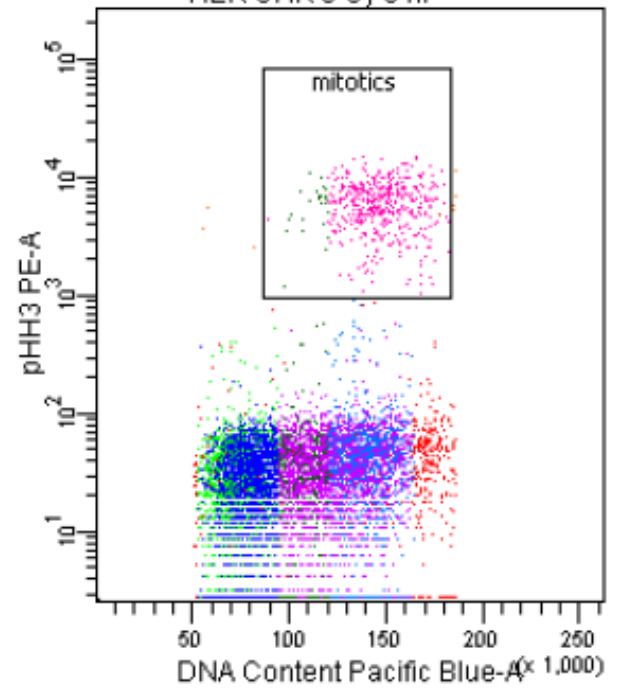
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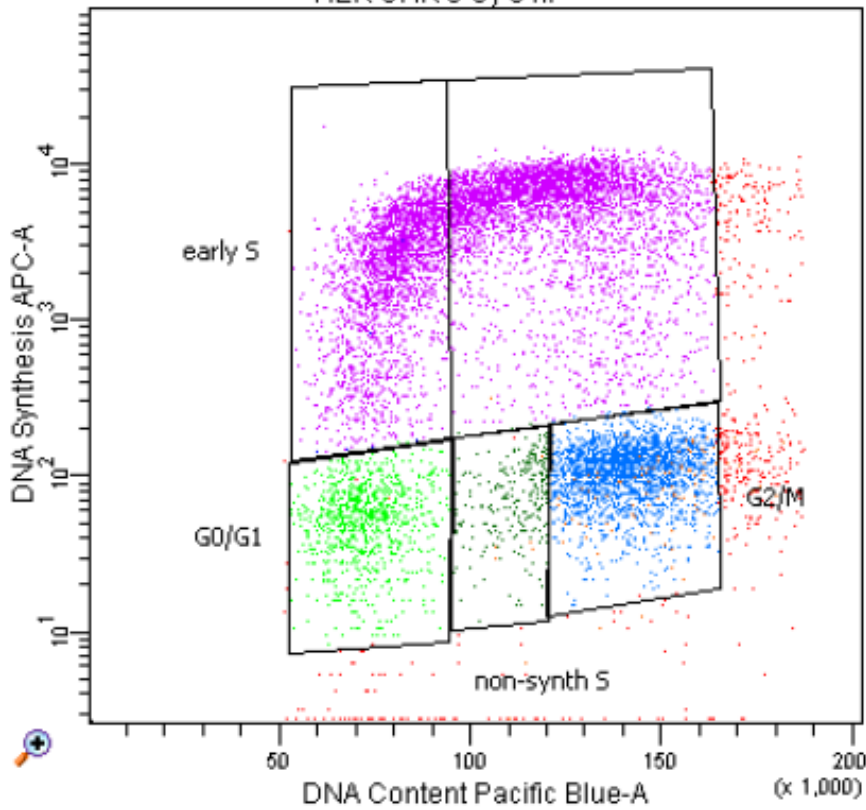
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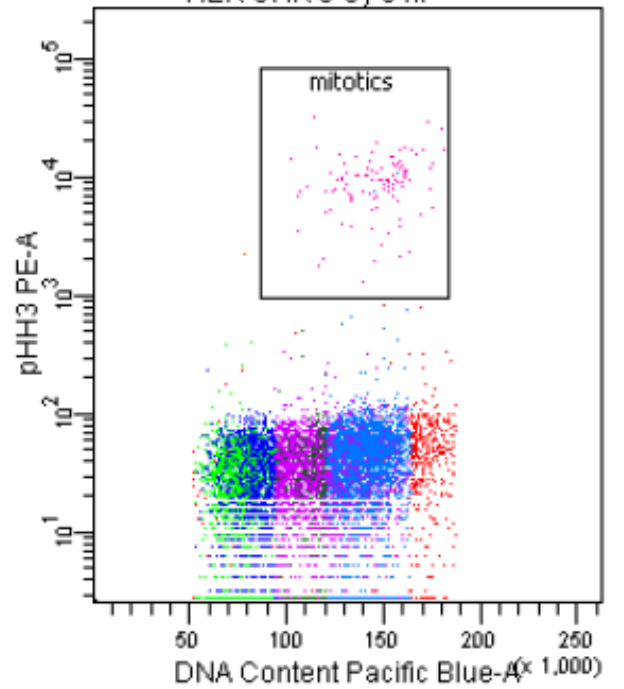
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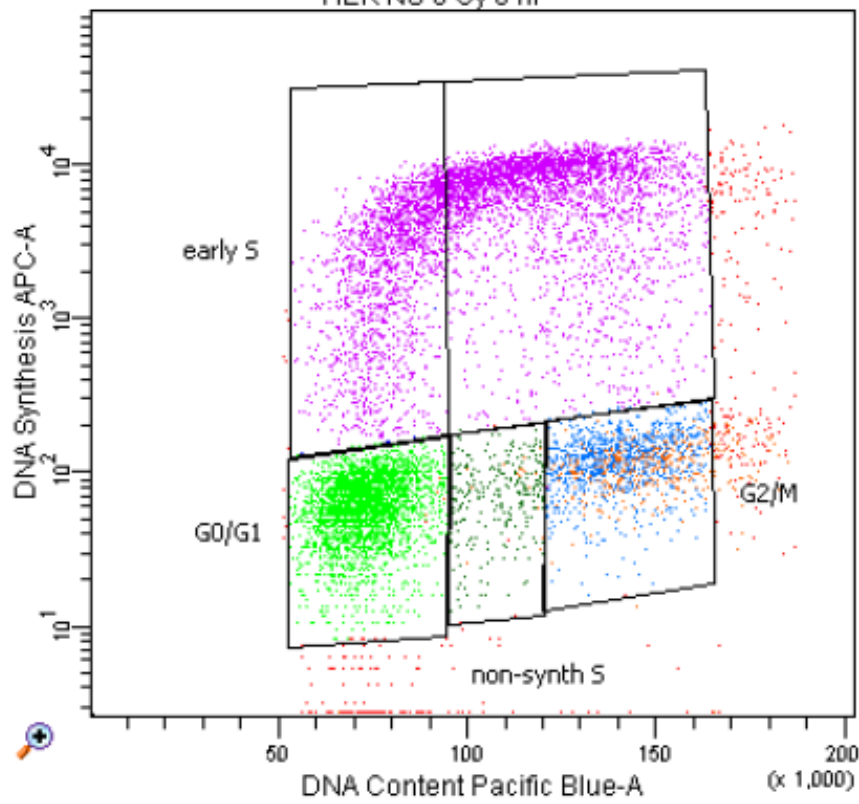
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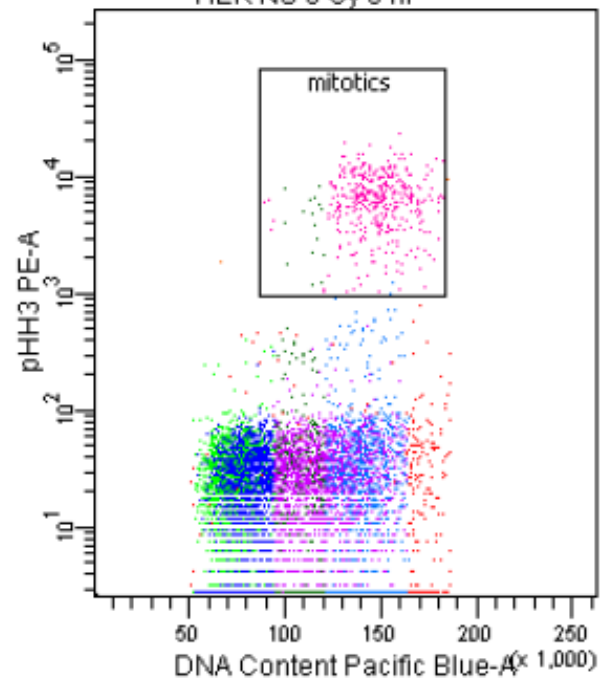
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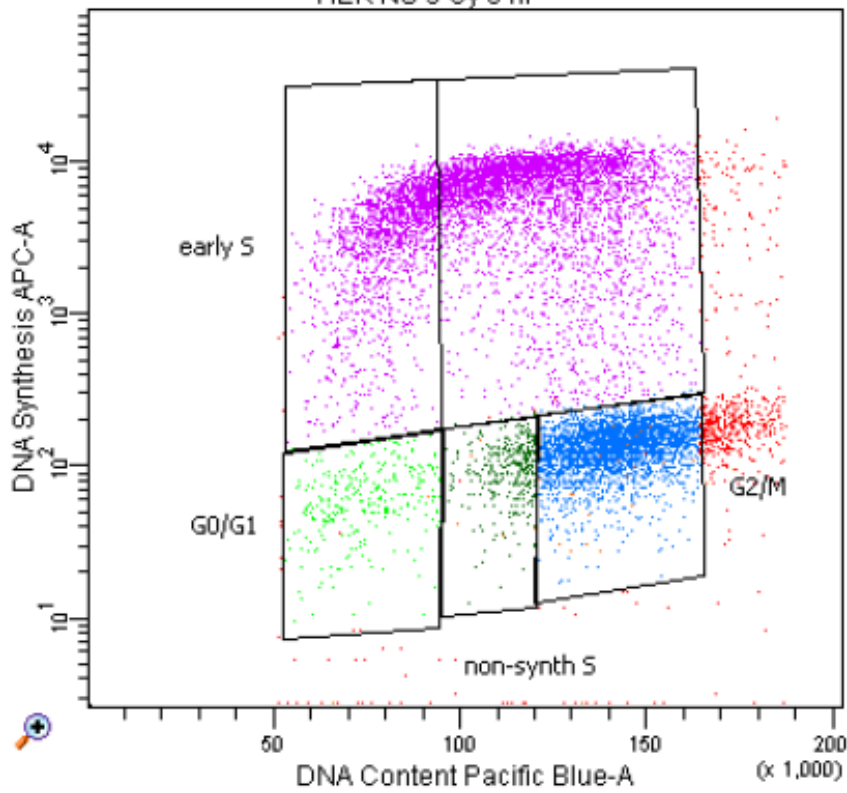
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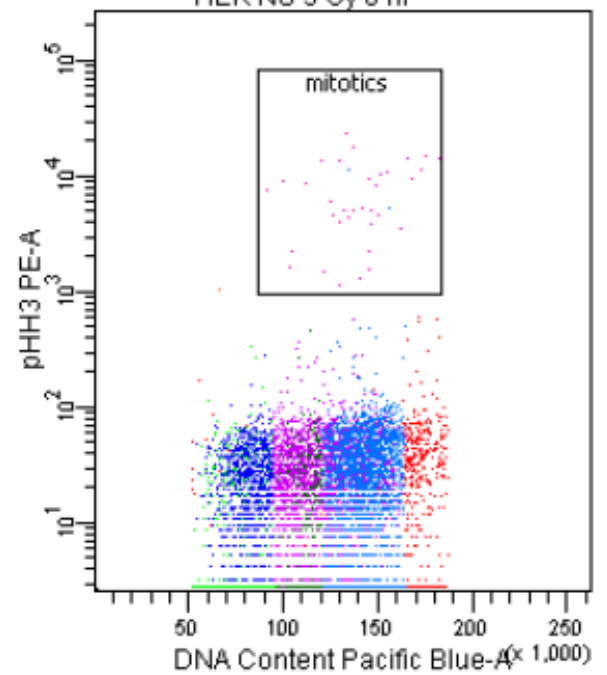
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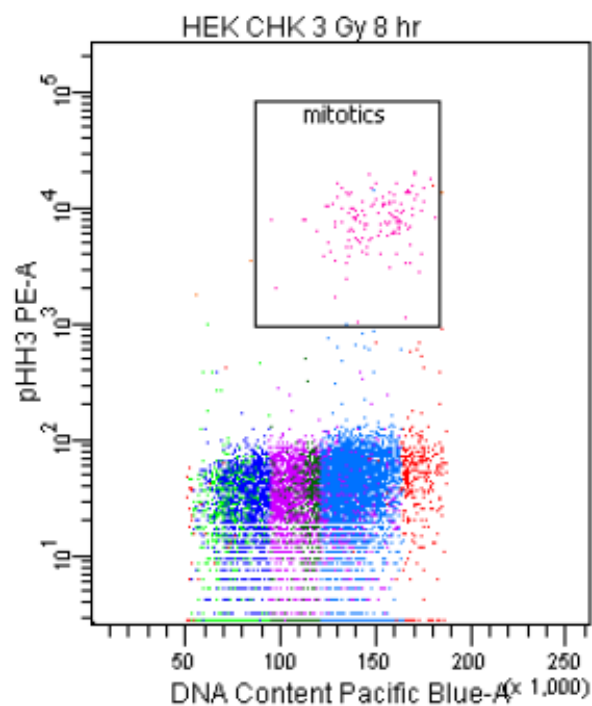
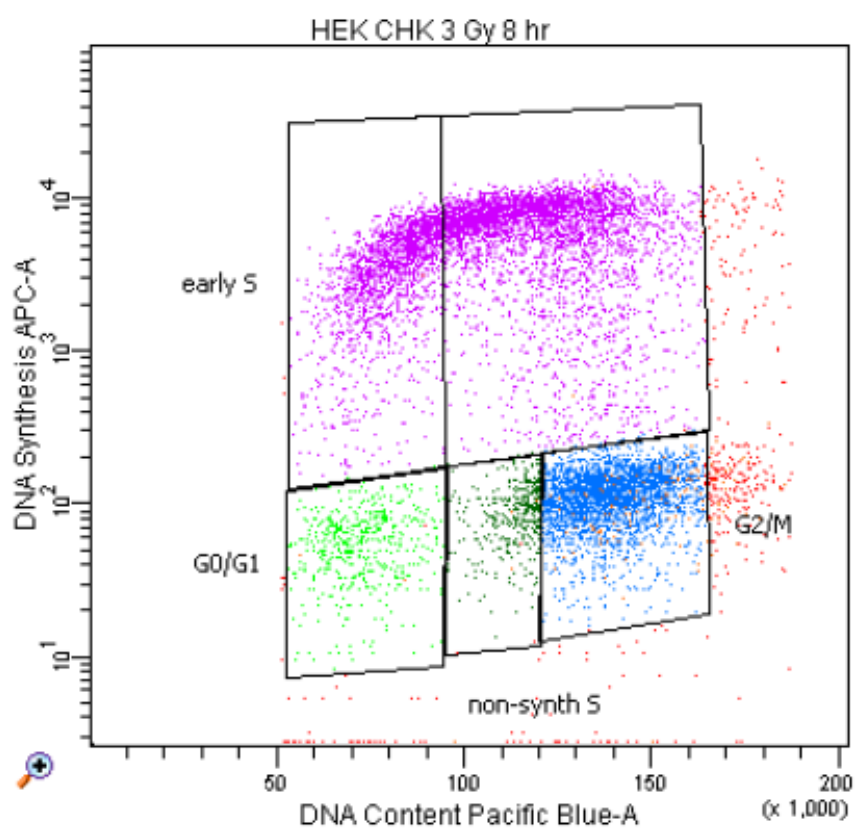
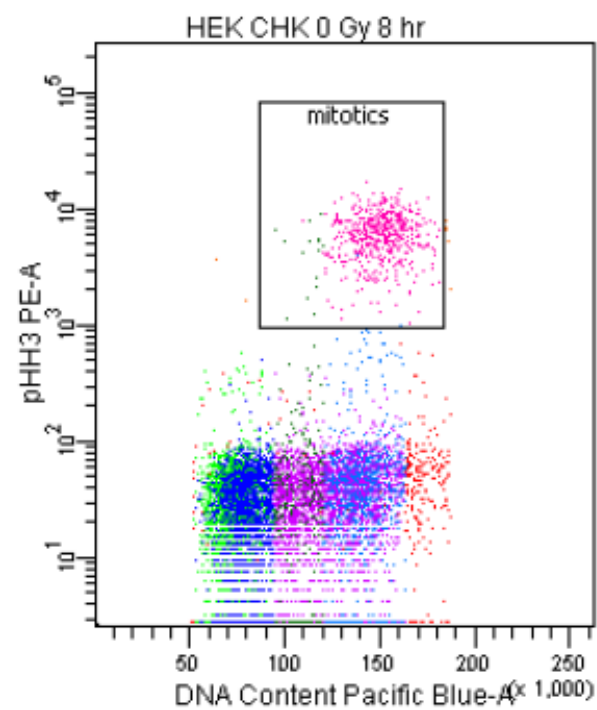
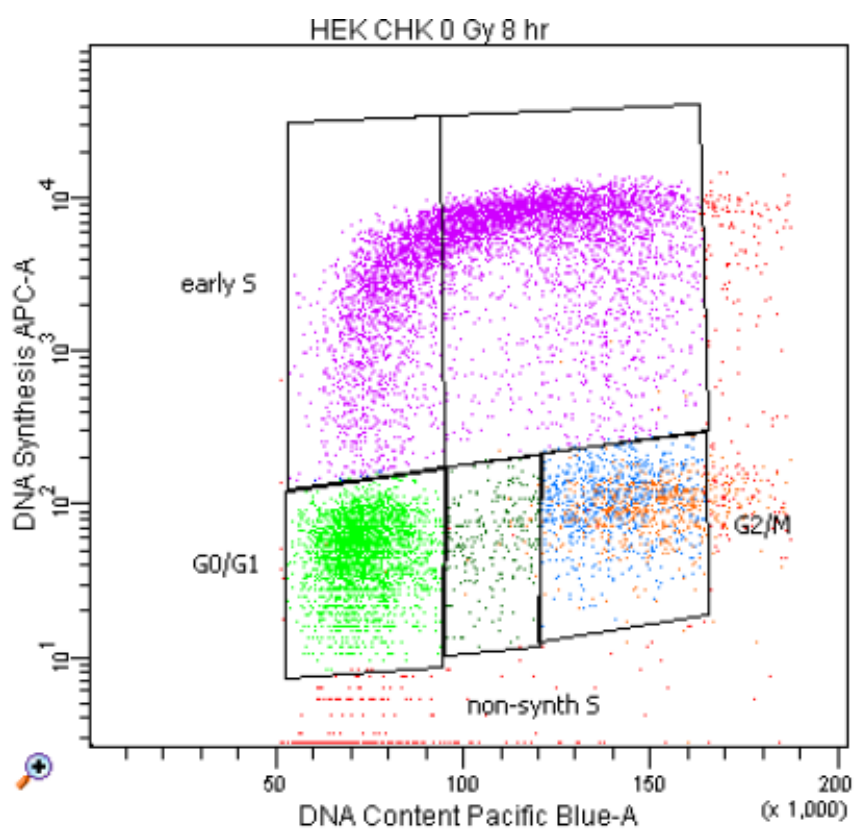


HEK NS 3 Gy 8 hr



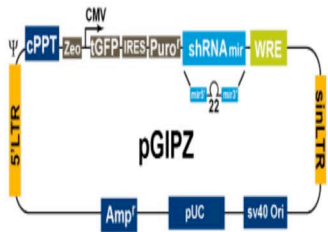
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Supplemental Figure 1.

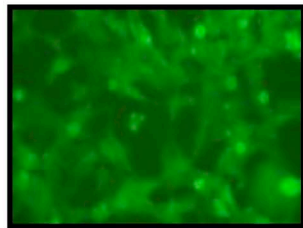
A.



B.



C.



A. pGIPZ plasmid structure (Open BioSystems).

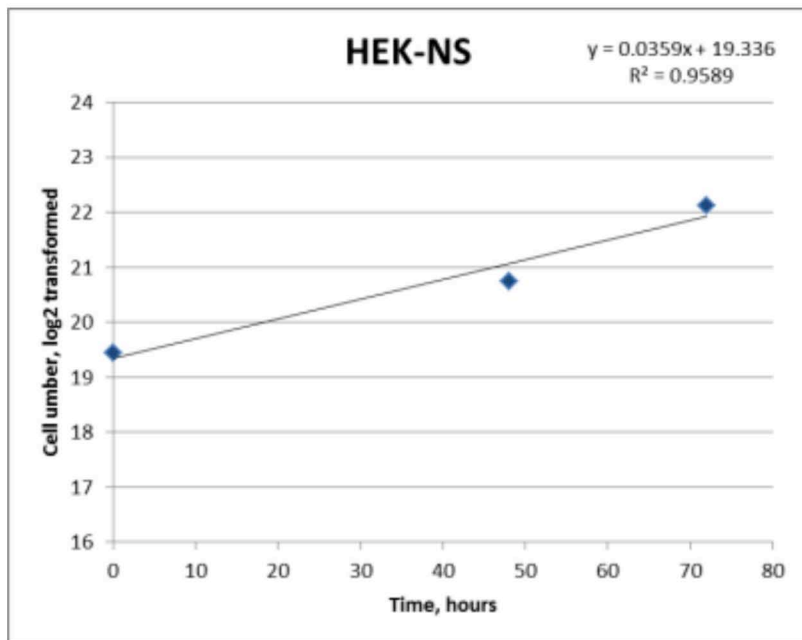
B. Image of HEK293 cells transduced with lentiviral particles (bright field)

C. Turbo GFP expression in lentivirally transduced HEK293 cells.

Note: Panels B and C are pictures of the same field of cells.

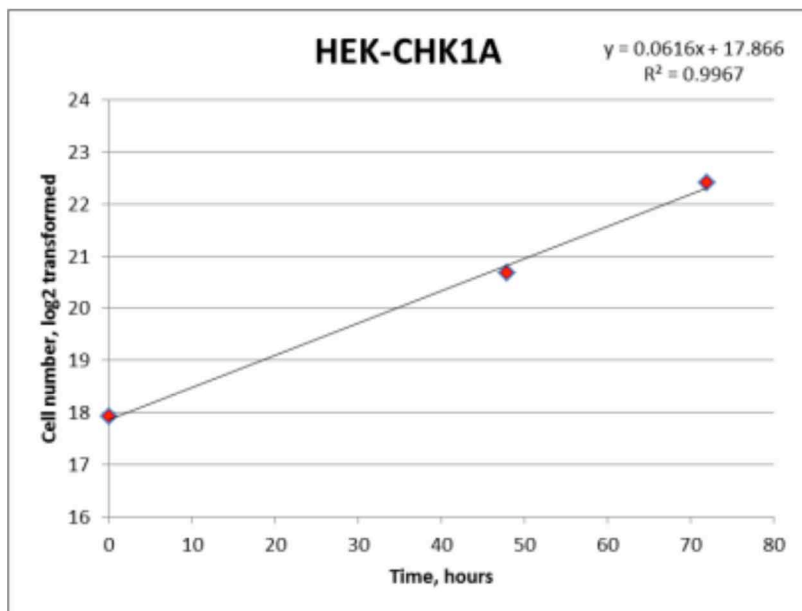
Supplemental Figure 2.

A.



Extrapolated doubling time:
 $1/\text{slope} = 1/0.0359 = 27.85$ hours

B.

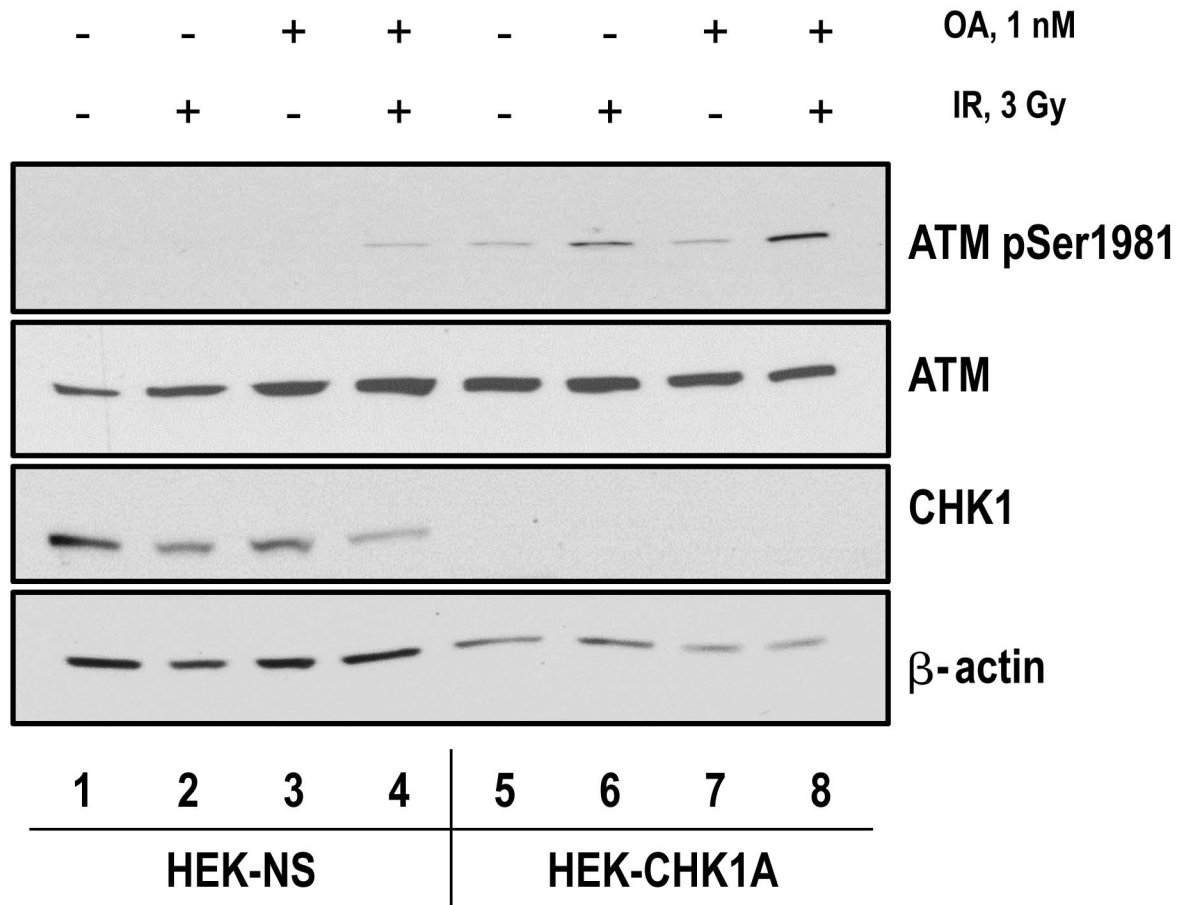


Extrapolated doubling time:
 $1/\text{slope} = 1/0.0616 = 16.23$ hours

Growth curves for HEK-NS and HEK-CHK1A stable cell lines.

Average cell numbers for each time point were log transformed to establish the linear growth range. For time-points in the linear growth range, data were log₂ transformed, plotted, and fitted with a linear trendline. The doubling time is the inverse of the slope ($y = ax + b$).

Supplemental Figure 3.

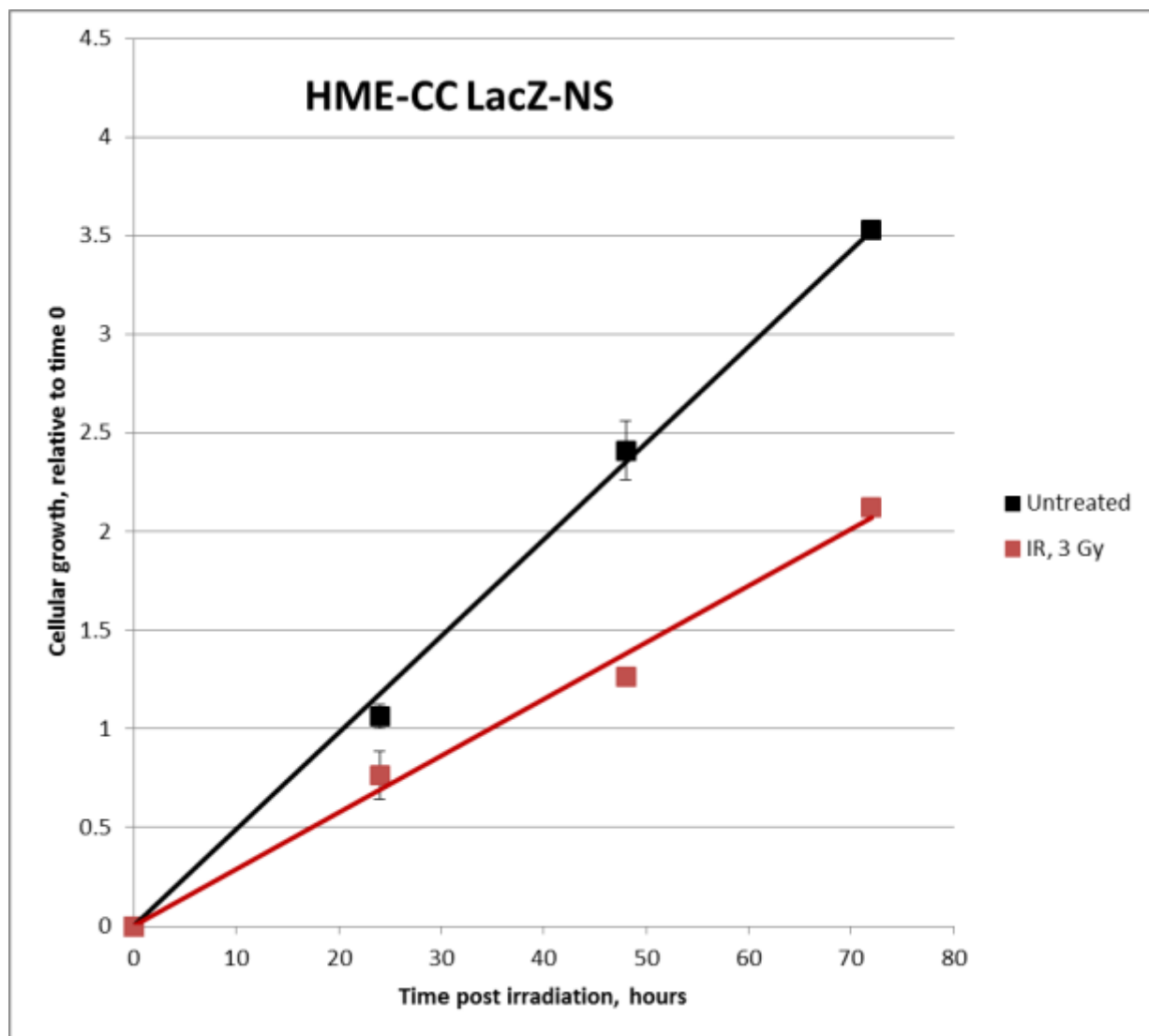


The cells were treated with either vehicle or okadaic acid (1 nM) following irradiation at 3 Gy. The samples were collected at 24 hours post irradiation and analyzed by Western blot.

Supplemental Figure 4.

Growth curves for HME-CC stable cell lines. Average cell numbers for each time point were log transformed to establish the linear growth range. For time-points in the linear growth range, data were log₂ transformed, plotted, and fitted with a linear trendline. The doubling time is the inverse of the slope ($y = ax + b$). Time 0 point represents the average number of cells prior to irradiation, which was set to 1 ($\log_2(1) = 0$). **(A)**. Control cell line, LacZ-NS. **(B)**. ATM-NS, ATM-deficient cell line. **(C)**. LacZ-CHK1A, CHK1-deficient cell line. **(D)**. Double-deficient cell line, ATM-CHK1A.

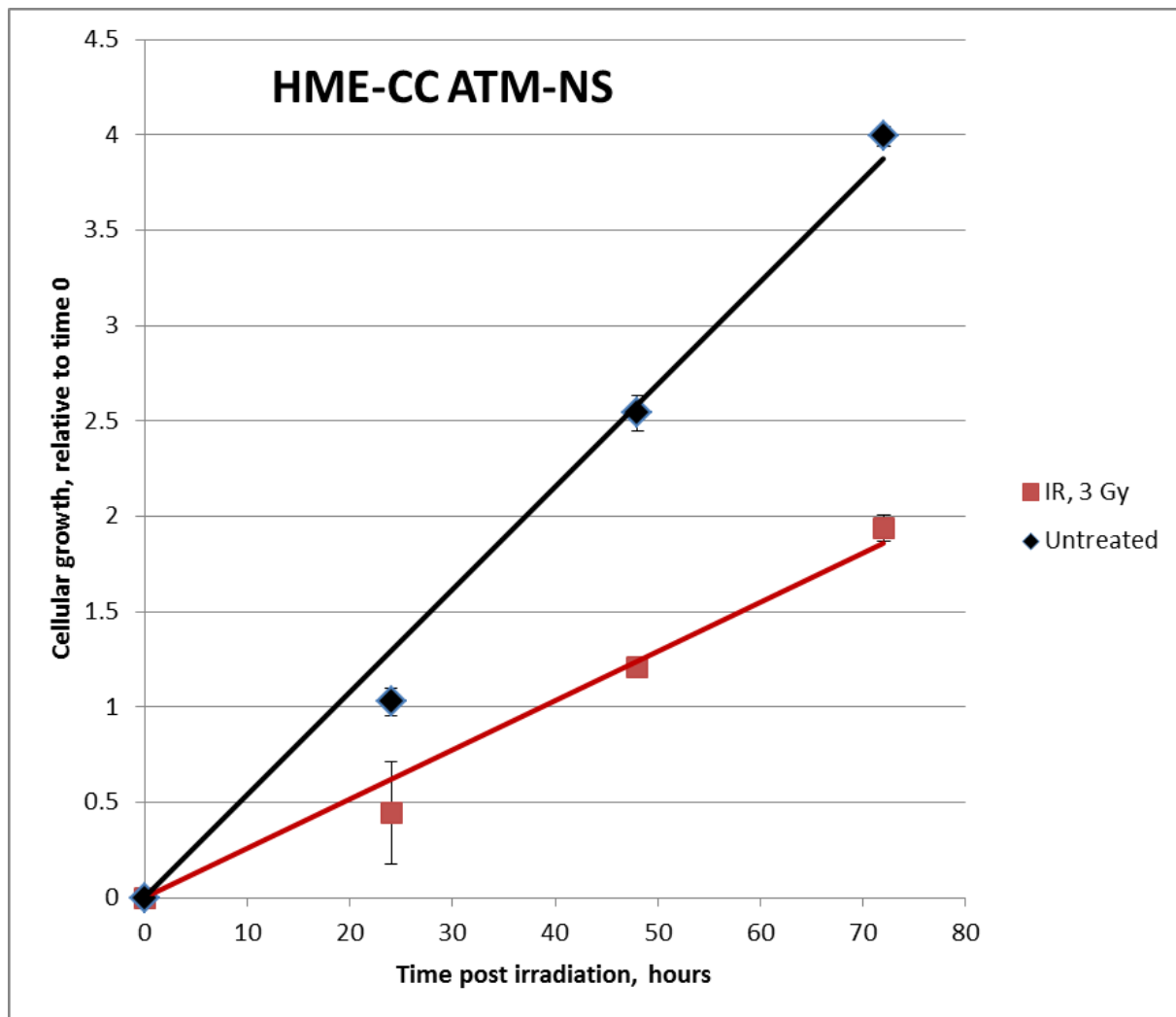
A.



Condition	Equation	R ²	Doubling time, hours
Untreated	$y = 0.049x$	0.9977	20.4
IR, 3 Gy	$y = 0.0287x$	0.9907	34.8

Supplemental Figure 4.

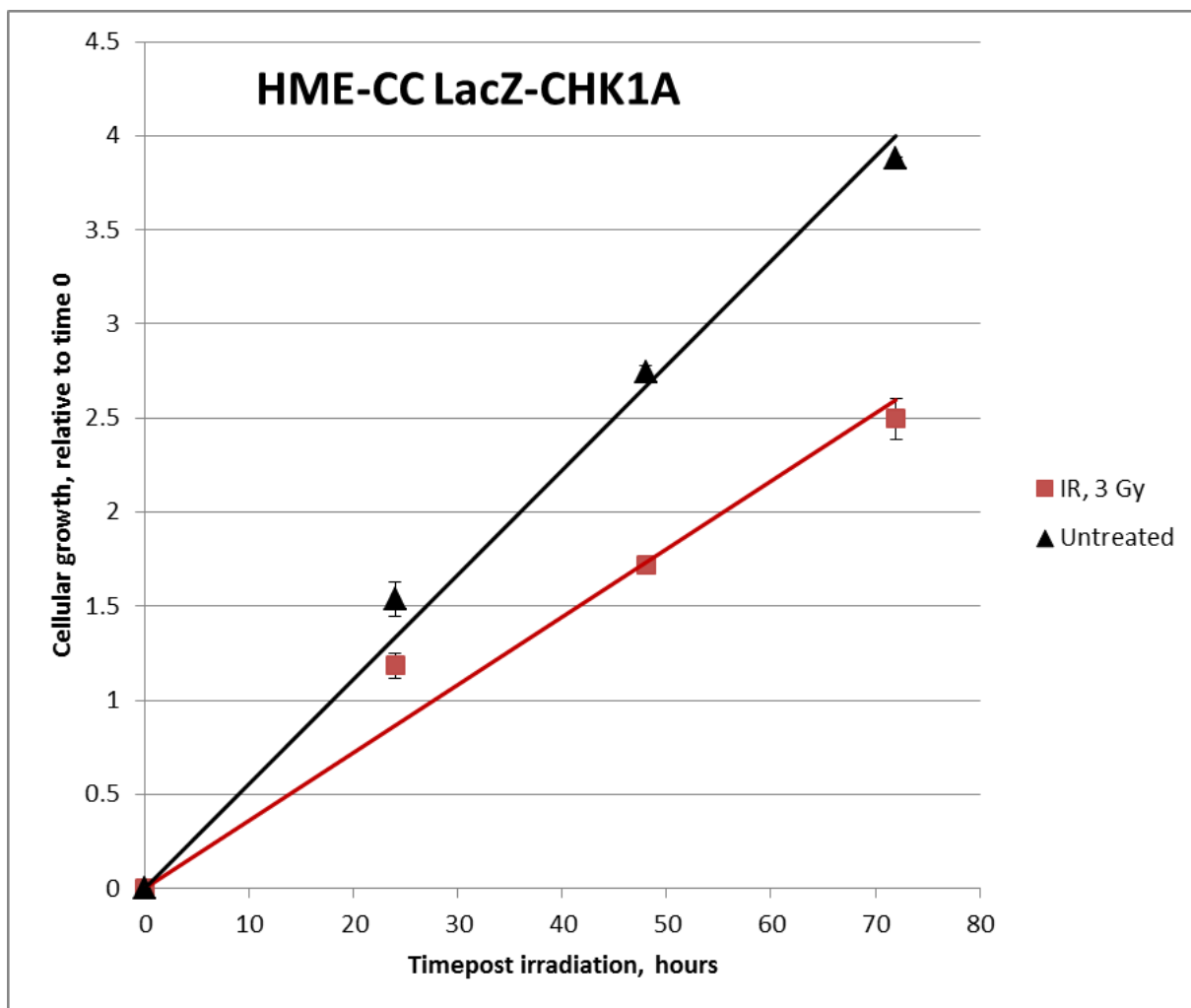
B.



Condition	Equation	R ²	Doubling time, hours
Untreated	$y = 0.0538x$	0.9906	18.6
IR, 3 Gy	$y = 0.0258x$	0.9826	38.8

Supplemental Figure 4.

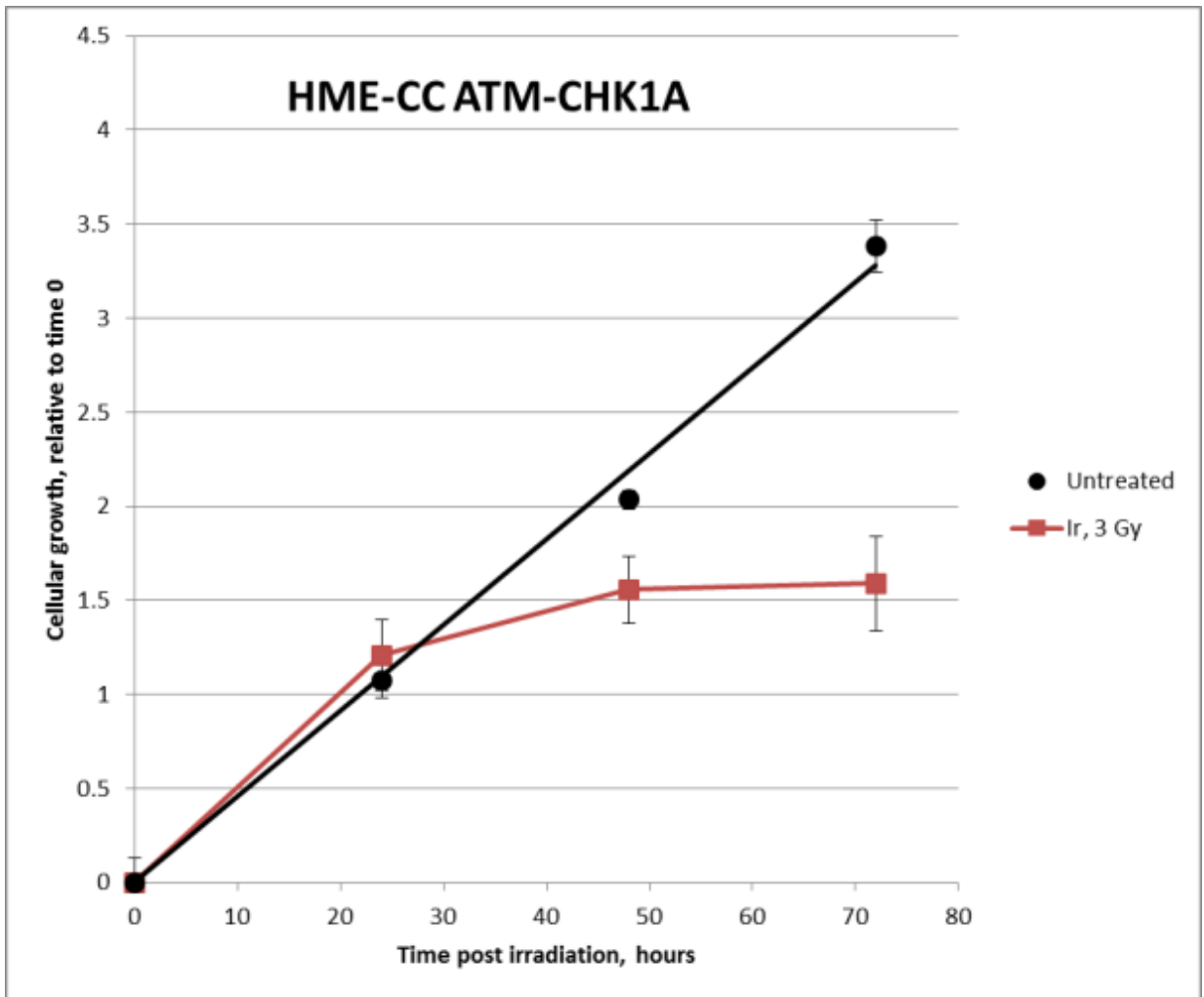
C.



Condition	Equation	R ²	Doubling time, hours
Untreated	$y = 0.0556x$	0.9925	18.0
IR, 3 Gy	$y = 0.0361x$	0.9664	27.7

Supplemental Figure 4.

D.



Condition	Equation	R ²	Doubling time, hours
Untreated	$y = 0.0556x$	0.9925	22.0
IR, 3 Gy	$y = -0.003x^2 + 0.0341x + 0.546$	1.0	N/A

Note: In the case of the double-deficient mutant, for the first 24 hours post IR, growth is linear and does not differ from the control condition; if extrapolated from the subsequent time-points (48 h and 72 h), the doubling time would equal 125 hours.

Supplementary Table 1 (Raw data for Figure 3A). Measured percentages of cells in mitosis in control and following irradiation (3 Gy) at the indicated time points.

Biological replicate	HEK-NS cell line							
	Non-irradiated (control)				Irradiated, 3 Gy			
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h
Set 1	3.2	4.0	3.3	-	0.4	0.4	0.3	-
Set 2	3.4	4.4	3.8	3.7	0.3	0.4	0.2	0.3
Set 3	2.7	3.8	4.0	3.6	0.2	0.1	0.2	0.3
Set 4	3.7	3.8	3.9	4.0	0.5	0.3	0.3	0.3
Set 5	4.2	4.1	-	-	0.5	0.4	-	-
Set 6	2.3	2.5	2.8	-	0.6	0.4	0.3	-
Set 7	3.1	3.2	4.0	-	0.4	0.2	0.3	-
Set 8	4.1	4.0	4.7	-	0.4	0.3	1.3	-
Set 9	2.4	2.2	2.5	-	0.6	0.3	0.4	-
Average	3.2	3.6	3.6	3.8	0.4	0.3	0.4	0.3
SEM	0.23	0.25	0.25	0.12	0.04	0.04	0.13	0.01
Biological replicate	HEK-CHK1A cell line							
	Non-irradiated (control)				Irradiated, 3 Gy			
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h
Set 1	2.7	4.0	3.5	-	0.8	1.1	1.2	-
Set 2	4.7	5.0	5.0	5.0	0.4	0.9	1.2	1.5
Set 3	3.2	3.8	5.0	4.6	0.5	0.6	1.5	1.7
Set 4	4.6	4.8	4.9	5.3	1.1	0.8	1.0	1.2
Set 5	3.3	4.5	-	-	1.2	1.4	-	-
Set 6	4.0	4.4	-	-	1.1	1.2	-	-
Set 7	3.8	4.6	4.5	-	0.6	1.0	1.7	-
Set 8	4.5	4.7	3.9	-	0.6	1.1	0.5	-
Set 9	2.3	2.4	3.8	-	1.2	0.9	1.2	-
Average	3.7	4.2	4.4	5.0	0.8	1.0	1.2	1.5
SEM	0.29	0.26	0.24	0.20	0.11	0.08	0.13	0.15

Supplementary Table 2. Quantification of Western blot data from Figure 5B.

Lane #	Condition	Band densitometry				Normalized to total CHK1 protein			Expressed as fold change relative to untreated CHK1-proficient control (lane 1)		
		CHK1 total	pSer345	pSer317	pSer296	pSer345	pSer317	pSer296	pSer345	pSer317	pSer296
Lane 1	HEK-NS Control	158158	146939	55636	123088	0.93	0.35	0.78	1.0	1.0	1.0
Lane 2	HEK-NS Irradiated	123816	205978	188371	88182	1.66	1.52	0.71	1.8	4.3	0.9
Lane 3	HEK-CHK1A Control	5811	233118	6279	4055	40.12	1.08	0.70	43.2	3.1	0.9
Lane 4	HEK-CHK1A Irradiated	1342	246094	38908	66258	183.38	28.99	49.37	197.4	82.4	63.4